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VOL. 18, SEC. C. NOVEMBER, 1940 NUMBER 11

CONTRIBUTIONS TO A STUDY OF THE FUNGUS FLORA OF NOVA SCOTIA

V. DISCOMYCETES[1]

BY LEWIS E. WEHMEYER[2]

Introduction

The species of Discomycetes presented in this list undoubtedly represent only a small fraction of the flora of the region. Only one summer, that of 1931, was sufficiently moist to yield a representative crop of fleshy forms. During that season a large representation of the Geoglossaceae were found in abundance, whereas only a few scattered collections were obtained in the other, drier, summers. Most of the species of the Helvellaceae and Pezizaceae were also collected during this same summer. The collections made in the remaining seasons were of a scattered nature, obtained in the course of general collecting.

The present arrangement of the Discomycetes is in such an unsettled and incomplete state that no attempt has been made to follow the latest arrangements and taxonomy. The nomenclature followed, in general, is that of Rehm (10).

The localities in which the majority of the collections were made have been described in the first paper of this series (14) and are referred to here merely by name.

The writer wishes to take this opportunity to thank the specialists in various groups who have so kindly aided him in the determination of many of these collections.

DISCOMYCETES

Exoascaceae

Taphrina caerulescens (Mont. & Desm.) Tul. On leaves of *Quercus* sp. Pictou Beach, Pictou Co., July 22, 1931, (1113).

— **communis** (Sadeb.) Gies. On fruits of *Prunus americana*, Bedford, Halifax Co., July 2, 1929, leg. A. R. Prince, (15).

— **Robinsoniana** Gies. On female catkins of *Alnus incana*, Salmon River, July 14, 1931, (1110). Asci from this material were somewhat larger (31–53 × 8.8–10.6 μ) than the measurements given by Ray (9, p. 58), but they are apparently this species.

[1] *Manuscript received March 7, 1940.*

Contribution from the Department of Botany, University of Michigan, Ann Arbor, Michigan. Papers from the Department of Botany, University of Michigan, No. 715.

[2] *Associate Professor of Botany, University of Michigan.*

Elaphomycetaceae

Elaphomyces muricatus Fr. In hole in tree, New Glasgow Rd., July 25, 1931, (1135); surface of ground, Mt. Thom, Aug. 10, 1931, (1135a), both det. by C. W. Dodge. No. 1135 was gnawed by squirrels and no doubt both specimens were carried by squirrels or other rodents.

Operculatae

Helvellaceae

Gyromitra esculenta Fr. On soil, Glenholme, Colchester Co., June 10, 1925, leg. A. R. Prince, (1098); Folleigh Lake, Oct., 1928, leg. A. R. Prince, (236). Seaver (13, p. 252) considers this as merely a gyrose form of *Elvela infula* Schaeff., which may well be true, but these collections are so distinct in form from No. 1416 that they are kept separate here.

—**(Helvella) infula** (Schaeff.) Quél. On duff under conifers, Upper Brookside, Aug. 27, 1931, leg. A. H. Smith, (1416). Stems 7–10 cm. in length, pileus typically saddle-shaped.

Helvella crispa (Scop.) Fr. On old stump, Earltown Rd., Aug. 22, 1931, (1387).

— **elastica** Bull. On soil, mixed woods, Earltown Rd., Aug. 21, 1931, (1378).

— **lacunosa** Afzel. (*H. Mitra* L.). Among moss, Upper Brookside, July 4, 1931, (381). Mixed woods, Earltown Rd., Aug. 21, 1931, (381a). The stalk of 381a dried a yellowish colour. All gradations of stipe colour seem to occur.

Macropodia macropus Fckl. On rotten logs and moss, Upper Brookside, July 17, 1931, leg. A. H. Smith, (1075); Lake O'Law, Inverness Co., Aug. 6, 1931, (1075a); Mt. Thom, Aug. 10, 1931, leg. A. H. Smith, (1075b). The large central oil globule in the spores of this and the following species of *Macropodia* would place them in the genus *Helvella* as circumscribed by Nannfeldt (6).

— **platypodia** Dodge. On soil, Upper Brookside, July 18, 1931, leg. A. H. Smith, (1080) det. F. J. Seaver.

— **subclavipes** (Phill. & Ell.) Rehm. On moist soil, Victoria Park, (240).

Pezizaceae

Ascophanus carneus (Pers.) Boud. On dung (bear ?), Upper Brookside, July 24, 1931, (1498). Obtained in gross culture. The apothecia have the colour and mycelial strands of *A. testaceus*, but the smaller fruit bodies and rough spores of *A. carneus*. The tips of the paraphyses are broader (4.5–5.5 μ below; 10–14 μ above) than given for *A. carneus*.

— **granulatus** (Bull.) Speg. forma **cervorum** Rehm. On cow dung, Salmon River, Aug. 1, 1931, (1204). This collection is intermediate between *A.* (*Humaria*) *granulatus* and *A. granuliformis* (Crouan) Boud. It has the long asci (80–140 $\times$ 17–23 μ), larger apothecia, and yellowish granular paraphyses of the former, but the smaller spores of the latter. The forma *cervorum*, with many spores 10 μ long, mentioned by Rehm (10, p. 943), seems to be the closest named form.

— **lacteus** (Cke. & Phill.) Phill. On cow dung, Upper Brookside, July 3, 1931, (377). The white, or yellowish-white, species of Ascophanus, *A. argenteus*, *A. lacteus*, *A. microsporus*, and *A. ochraceus* are very much confused and no two authors seem to agree on all characters. This collection agrees best with Rehm's description of *A. lacteus* (10, p. 1086).

Geopyxis catinus (Holmsk.) Sacc. On soil under spruce, Upper Brookside, Aug. 20, 1931, leg. A. H. Smith, (1373).

Humaria xanthomela Cke. var. **americana** (Rehm) Sacc. & Trott. On mossy soil at base of beech, Upper Brookside, Sept. 3, 1931, (1460), det. E. K. Cash.

Lamprospora polytrichina (Rehm) Seaver. On soil, and among mosses, Valley, Colchester Co., July 1, 1929, (8); Salmon River, July 3, 1931, (367). The spores of these collections (11.5–14 μ in diameter) are larger than those given by Rehm (9–10 μ) but agree with the measurements of Cooke, Phillips, and Seaver.

Lasiobolus equinus (Müll.) Karst. On rabbit dung, Salmon River, July 3, 1931, (362).

Otidea leporina (Batsch) Fckl. Decayed wood, Salmon River, Aug. 18, 1931, leg. A. H. Smith, (1344); on soil, Upper Brookside, Aug. 20, 1931, leg. A. H. Smith, (1344a); on humus, Earltown Rd., Aug. 21, 1931, leg. A. H. Smith, (1344b).

Patella (Lachnea) albospadicea (Grev.) Seaver. On bare soil, Salmon River, July 3, 1931, leg. A. H. Smith, (365); North River Rd., Colchester Co., July 7, 1931, (418); Upper Brookside, July 10, 1931, leg. A. H. Smith, (418a), (418b); Upper Brookside, July 27, 1931, (418c). The spores of these collections, in general, run longer (21–24 × 8–11 μ), and in 418b broader (14 μ), than given by Seaver (13, p. 179). The apothecia are often covered by a white cobwebby mycelium.

Lachnea alpina (Fckl.) Sacc. On moose dung, Mt. Thom, Aug. 10, 1931, (1254); on porcupine dung, Mt. Thom, Aug. 10, 1931, (1254a). This is similar to, and has the stellate hairs of *L. stercorea*, but the asci (150–200 × 9–10 μ), spores (12–14 × 7–8 μ), and apothecia (0.5–2 mm.) are definitely smaller. It approaches *Humaria alpina* Fckl. as described by Cooke (1, p. 81), but Fuckel (3, Nachtr. 3:32) gives the spores of this species as 16 × 9 μ and says it differs from *L. stercorea* in its colour and the stellate hairs. If *L. alpina* does differ in the smaller spores and apothecia, this is it.

— **coprinaria** (Cke.) Sacc. On cow dung, North River Rd., Colchester Co., July 7, 1931, leg. A. H. Smith, (419); Upper Brookside, Sept. 7, 1931, (419b); on bear dung, Mt. Thom, Aug. 10, 1931, (419a).

— **erinaceus** (Schw.) Sacc. On wet sticks, Jollytown Rd., Colchester Co., July 26, 1929, (79).

According to Seaver, this species differs from *Patella albospadicea* only in its occurrence on wood. The spores of this collection are slightly smaller than those placed in *P. albospadicea*, but fit better Seaver's measurements for that species.

— **hemisphaerica** (Wigg.) Gill. On moss, Lake O'Law, Inverness Co., Aug. 6, 1931, (1220); Wentworth Valley, Cumberland Co., Aug. 29, 1931, (1220a).

— **scutellata** (L.) Gill. On decayed wood, Salmon River, July 3, 1931, (366); Folleigh Lake, July 20, 1931, (366a).

— **setosa** (Nees) Phill. On *Betula*, Upper Brookside, July 4, 1931, (387).

— **stercorea** (Pers.) Gill. On cow dung, Upper Brookside, July 3, 1931, (376). This collection has the stellate hairs of the var. *gemella* Karst. Rehm (10, p. 1057) gives *Humaria alpina* Fckl. as a synonym of this variety. The collections placed under *L. alpina* in this paper have smaller spores. The spores of this collection are 17–19 × 8.5–9.5 μ.

— **umbrorum** (Fr.) Gill. On bare soil, St. Peters, Richmond Co., Aug. 3, 1931, (1237). There is little to choose from between the descriptions of *L. hirta*, *L. scutellata* and *L. umbrorum*. This collection is placed in the latter species on account of the larger roughened spores, the shorter, sparser hairs and the occurrence on soil. The spores of this collection measure 21–24.5 × 11–12 μ.

Peziza ampelina Quél. On rocky bank, Upper Brookside, July 4, 1931, (389). These plants have the curved violet-brown paraphyses of *P. viridaria* and *P. violacea* and the small apothecia (5–12 mm.) of the former. The spores (14–17 × 7.5–9.5 μ) are, however, larger than those given for either of these two species. They come nearest to *P. ampelina*, although the apothecia are smaller and lighter in colour than given for that species.

— **atrovinosa** Cke. & Ger. On soil, Salmon River, Aug. 18, 1931, leg. A. H. Smith, (1348). This collection is characterized by the small apothecia, 1.5–4 cm. in diameter, which become expanded but not repand. They have a dark brown hymenium which becomes intense black at maturity. The asci are cylindric, 200–250 × 11–12 μ and turn blue with potassium iodide. The spores are broad-ellipsoid, coarsely tuberculate-ridged, hyaline and 12.5–14 × 8.5–9 μ. The paraphyses are terete, simple or slightly branched, 3.5 μ in diameter and expanded above (6–7 μ). It is related to *P. badia* but has smaller apothecia with a very dark hymenium and has smaller spores. It seems to fit best the above species. It is also near *P. chlamydospora* E. & E.

— **badia** Pers. On soil, Upper Brookside, July 26, 1929, (132); July 27, 1929, (174); July 18, 1931, leg. A. H. Smith, (295b); Sept. 3, 1931, leg. A. H. Smith, (1030b). On moss-covered wood, Victoria Park, June 24, 1931, (295).

The only apparent difference between *P. badia* and *P. brunneo-atra* is the larger apothecia of the former. The Nova Scotian plants placed in these two species showed all intergrading apothecial sizes, but they all dried a dark black and had hyaline to pale yellow-brown, roughened spores measuring 15–19 × (7)8–10 μ. The paraphyses were septate and yellow-brown except in Nos. 1030a and 1030b, where they were sparse, hyaline, and non-septate. Nos. 132 and 295b showed the olive colour of the hymenium given for *P. cervina* Fckl. The plants placed under *P. badia* include those with larger apothecia, 2.5–4 cm. in diameter.

— **brunneo-atra** Desm. On soil, Salmon River, July 15, 1931, leg. A. H. Smith, (295a); Victoria Park, July 23, 1931, (1030a). On sawdust, Salmon River, July 14, 1931, (1030). The collections placed here had apothecia 12–25 mm. in diameter.

— **fimeti** (Fckl.) Seaver. On cow dung, Upper Brookside, July 7, 1931, (378).

The spores of these plants (17.5–19 × 8.5–9.5 μ) are larger than the measurements of Seaver (8 × 16 μ) but agree with those of Rehm (15–18 × 9–10 μ).

— **Howsei** Roze & Boud. On soil, Upper Brookside, Aug. 16–20, 1931, leg. A. H. Smith, (1343, 1343b, 1343c); Sept. 3, 1931, (1343d). On decayed log, Salmon River, Aug. 18, 1931, (1343a). The collections placed here have been difficult to determine. In general, the presence of violet shades in the hymenium or, at least in the hypothecium, suggests *P. violacea*. They do not have the violet, curved paraphyses of that species, and the spores are definitely roughened at maturity, however. Seaver (13, p. 226) gives the spores of *P. violacea* as "sculptured," but these collections have spores definitely larger than *P. violacea* (10–14 × 5–9 μ, after various authors). These plants have a white punctate exterior and dry translucent or furfuraceous yellow-brown, with the appearance of a dried peach. They seem to fit best Roze and Boudier's *P. Howsei* (11). An inclusive description follows.

Apothecia 0.8–5 cm. in diameter, at first globose then cup-shaped to repand or undulate, often with a short stipe-like base. Young plants a clear pale lavender, becoming darker, eventually dull yellow-brown or olivaceous due to the browning of the hymenium. Lavender tints usually present in the hypothecium. Exterior white, punctate, furfuraceous. Asci cylindric, 250–300 × 14–17 μ, blue with potassium iodide. Spores hyaline, ellipsoid, biguttulate, somewhat roughened at maturity, 14–19 × 7–10 μ. Paraphyses sparse, simple, filiform with slightly enlarged tips, 3.5–4.5 μ in diameter.

The purple tints were mostly lacking in 295c, but the appearance of the dried plants was similar to all of the other collections, which is quite distinct from the dried plants of *P. badia*.

— **pustulata** (Hedw.) Pers. On soil, Upper Brookside, Aug. 18, 1931, leg. A. H. Smith, (1351). Apparently immature; paraphyses hyaline and not thickened at the apex. Hymenium brown with a violet cast.

— **repanda** Pers. On decayed log, Lake O'Law, Inverness Co., Aug. 6, 1931, (1223).

— **sepiatra** Cke.? On decayed sticks, Upper Brookside, July 10, 1931, leg. A. H. Smith, (481). Apothecia 1–2 cm. in diameter, soon repand, tough, externally dirty white with an olivaceous cast. Hymenium brown, smooth, Asci cylindric, 250–300 × 14–16 μ, blue with potassium iodide. Spores ellipsoid, hyaline, smooth, biguttulate, 19–22 × 10–13 μ. Paraphyses simple, apex clavately enlarged, 5–7 μ, somewhat curved or bent; tips filled with yellow-brown granules. This collection does not fit the description of any species exactly. It is lighter in colour than *P. sepiatra* and *P. clypeata*, has smaller spores than the latter, and has larger spores than *P. subrepanda*.

— **succosa** Berk. On bare soil, Victoria Park, Aug. 16, 1929, (242); Upper Brookside, July 10, 1931, (480).

— **sylvestris** (Boud.) Sacc. & Trott. On soil, Victoria Park, June 24, 1931, (294). This collection differs from No. 329 of *P. vesciculosa* only in the smaller apothecia (1–2 cm. in diameter) with less well developed stipitate base and in the smaller spores (17.5–19 × 10 μ). Rehm (10, p. 1018) places such material in *P. vesciculosa* var. *cerea* (Sow.) Rehm, but Seaver places them under *P. sylvestris*. This differs from *P. fimeti* only in the slightly lighter hymenium and the habitat.

Peziza vesciculosa Bull. On bare soil, Upper Brookside, June 29, 1931, leg. A. H. Smith, (329).

Typical of *P. vesciculosa*, but not giving a blue iodine test. Spores larger (19–23 × 10–11 μ) than in *P. sylvestris*.

Saccobolus Kerverni (Crouan) Boud. On dung (bear ?), Upper Brookside, July 24, 1931, (1497). Apothecia (200–400 μ) and spores (17–21 × 6.5–8.5 μ) somewhat smaller than given in descriptions.

Sphaerospora brunnea (Alb. & Schw.) Massee. Among moss on charred logs, Victoria Park, July 16, 1931, (406).

Inoperculatae

Geoglossaceae

Apostemidium fiscellum Karst. On submerged wood, Salmon River, Aug. 19, 1933, (1638).

Apothecia yellow-brown when fresh, turbinate, 1–2 mm. diameter, drying black with a crenulate margin. Excipulum of pale brown parenchyma with a dark brown cortex, 30–120 μ thick, thicker at the base than at the margin. Asci filiform, cylindric, 120–150 × 5–6 μ. Spores parallel, filiform, hyaline, 85–100 × 1 μ. Paraphyses filiform, simple, clavate thickened at the apex, 1.5 μ diam., hyaline.

The shorter asci and spores are the characters used to distinguish *A. fiscellum*, which is an European species not reported from America by Durand (2, p. 457).

Corynetes robustus Durand. On soil under hardwoods, Upper Brookside, Aug. 27, 1931, leg. A. H. Smith, (1426); Folleigh Lake, Aug. 29, 1931, (1426a). Both of these collections fit very well Durand's description except for the fact that the ascigerous portion is sharply delimited from the stem in these plants.

Cudonia circinans (Pers.) Fr. Among moss or on duff, usually under spruce or other conifers, Middle River, Victoria Co., Aug. 5, 1931, (1216) (1216a); Salmon River, Aug. 18, 1931 and Sept. 2, 1931, (1216d) (1216f); Upper Brookside, Aug. 12, 1931, (1216b), Aug. 17 and 20, 1931, leg. A. H. Smith, (1216c) (1216e). All of these collections showed numerous budding ascospores, to such an extent that many asci were completely filled with the small ellipsoid secondary conidia which are one-celled and 3.5–6 × 1.5–2 μ. The primary ascospores were long fusoid to clavate, 31–49 × 1.5–3 μ. Although not given in his description of *C. circinans*, these budding secondary conidia are mentioned elsewhere by Durand (2, p. 388).

— **lutea** (Pk.) Sacc. On leaves of *Fagus*, Upper Brookside, Aug. 14, 1931, (1304); Victoria Park, Aug. 17, 1931, leg. A. H. Smith, (1304a).

Geoglossum fallax Durand. Among moss under hardwoods, Lake O'Law, Inverness Co., Aug. 6, 1931, (1219).

— **glabrum** Pers. On soil, humus or much decayed wood, Westcook's Cove, Guysborough Co., leg. A. R. Prince, Sept. 5, 1925, (1159); Princeport, Colchester Co., Aug. 26, 1927, leg. A. R. Prince, (6111); Mt. Thom, Pictou Co., Aug. 11, 1927, leg. A. R. Prince, (6109); Victoria Park, July 23, 1931, (1130); Upper Brookside, July 24, 1931, (1130a); Folleigh Lake, Aug. 19, 1931, (1130b); Earltown Rd., Aug. 19, 1931, (1130c).

— **nigritum** Cke. On soil, Folleigh Lake, Aug. 18, 1927, leg. A. R. Prince, (6099); Earltown Rd., Aug. 19, 1931, leg. A. H. Smith, (1363). Collection No. 1363 contains minute plants, 1–2 cm. tall. The paraphyses are those of *G. nigritum* and the spores are 40–55 × 5–6 μ. This seems to be the dwarf, poorly developed form mentioned by Durand (2, p. 427) as being described as *G. glutinosum* var. *minus* Sacc. No. 6099 is somewhat intermediate between this species and *G. glabrum*. The paraphyses are typical of *G. nigritum* when young, but become more darkly coloured, more closely septate and somewhat moniliform in age. The spores measure 75–90 × 7.5 μ.

— **affine** Durand. On mossy soil, Wentworth Valley, Cumberland Co., Aug. 29, 1931, leg. A. H. Smith, (1436).

— **difforme** (Fr.) Durand. On decayed stump, Earltown Rd., Aug. 22, 1931, (1386); on duff and humus under hardwoods, Upper Brookside, Aug. 24, 1931, (1386a); Aug. 27, 1931, leg. A. H. Smith, (1386b); Wentworth Valley, Cumberland Co., Aug. 29, 1931, leg. A. H. Smith, (1386c).

— **glutinosum** (Pers.) Durand. On humus, Earltown Rd., Aug. 21, 1931, leg. A. H. Smith, (1381).

Leotia lubrica (Scop.) Pers. On wet soil, Princeport, Colchester Co., Aug. 26, 1927, leg. A. R. Prince, (6032). On conifer duff, Upper Brookside, Aug. 14, 1931, (440a).

— **lubrica** forma **Lloydii** (Rehm) Durand. Among moss under conifers, Victoria Park, Sept. 10, 1927, (440). Typical of Durand's (2, p. 447) description.

— **stipitata** (Bosc.) Schroet. Among moss on duff, Victoria Park, Sept. 10, 1927, (441); Salmon River, Aug. 18, 1931, leg. A. H. Smith, (441a).

Microglossum rufum (Schw.) Underw. On soil and duff, Upper Brookside, Aug. 26, 1927, leg. A. R. Prince, (6217); Economy River, Colchester Co., Aug. 31, 1927, leg. A. R. Prince (6112); Northeast Margaree, Inverness Co., Sept. 4, 1927, (439); Folleigh Lake, July 20, 1931, (1099); Earltown Rd., Aug. 19, 1931, (1099a).

— **viride** (Pers.) Gill. On soil, mixed woods, Wentworth Valley, Cumberland Co., Aug. 29, 1931, leg. A. H. Smith, (1432). These plants have the smooth fibrous stem of *M. olivaceum* but the spores are those of *M. viride* (17–23 × 5–6 μ).

Spathularia clavata (Schaeff.) Sacc. On soil or duff, Princeport, Colchester Co., Aug. 26, 1927, leg. A. R. Prince, (6222); Upper Brookside, Aug. 14, 1931, (63a); Sept. 9, 1931, leg. A. H. Smith, (63b).

— **velutipes** Cke. & Farl. On decayed wood and humus, Upper Brookside, Aug. 1927, leg. A. R. Prince, (6216); Killag Mines, July 30, 1931, (52a).

Trichoglossum Farlowii (Cke.) Durand. On soil under hardwoods, Wentworth Valley, Cumberland Co., Aug. 29, 1931, leg. A. H. Smith, (1435).

— **hirsutum** (Pers.) Boud. On soil, sandy creek, Wentworth Valley, Cumberland Co., Aug. 29, 1931, leg. A. H. Smith, (1437).

— **velutipes** (Pk.) Durand. On soil, mixed woods, Earltown Rd., Aug. 21, 1931, (1380).

Vibrissea truncorum (Alb. & Schw.) Fr. On submerged sticks in cold stream, Halifax Co., June 27, 1926, (P376).

Phacidiaceae

Coccomyces Cembrae Rehm. On stems of *Larix laricina*, Upper Brookside, July 13, 1931, (1004).

— **coronatus** (Schum.) Rehm. On dead leaves of *Fagus*, Upper Brookside, Sept. 3, 1921, (1467).

— **coronatus** var. **trigonus** (Schm. & Kze.) Karst. On dead leaves of *Fagus* and *Acer*, Upper Brookside, Aug. 11, 1931, (1277). On dead leaves of *Populus tremuloides*, Salmon River, Aug. 1, 1931, (1198). These collections differed from No. 1467 in the presence of definite rounded brown spots, definitely margined by a blackened zone, the fusoid character of the apothecia on these spots and the smaller spores (25–37 × 1–3 μ). The apothecia also have a raised ridge-like margin. A wide variation of form is found in exsiccati of *C. coronatus*, however, and apothecia of this same appearance are found in Syd., Myc. Germ. 1611, for instance. This is probably the var. *trigonus*, with shorter spores.

Coccophacidium Pini (Fr.) Rehm. On stems of *Pinus Strobus*, Victoria Park, Aug. 30, 1933, (1647).

Rhytisma Ilicis-canadensis Schw. On leaves of *Nemopanthus mucronata*, Great Anse, Richmond Co., Aug. 3, 1931, (1234); Upper Brookside, July 28, 1931, (1234a).

Stictidaceae

Ocellaria ocellata (Fr.) Schroet. On stems of *Populus* and *Salix* spp., Upper Brookside, July 29, 1929, (150); Aug. 2, 1929, (196); June 27, 1931, (303); Victoria Park, June 26, 1935, (1710).

The spores of these collections appear to run larger than measurements given for European collections. Most European citations run from 21–30 × 9–12 μ. Fuckel (3, Nachtr. 1 : 326) gives them as 40 × 12 μ. Wollenweber (15, p. 569) cites the spores in 8-spored asci as 23–28 × 9–12 μ, occasionally 20–40 × 8–14 μ, and those in 4–6-spored asci as 25–40 × 8–14 μ. The spores of these collections were (21)26–43 × (11.5)13–16 μ.

Propolis faginea (Schrad.) Karst. On bark and wood of *Fagus*, Upper Brookside, July 14, 1929, (57) June 28, 1931, (316); July 16, 1931, (1066); June 19, 1933, (1665); Victoria Park, Aug. 8, 1929, (218); Green Oaks, Colchester Co., July 9, 1931, (470).

Common, asci 90–106 × 12–15 μ, spores 20–26.5 × 5.5–7 μ.

— **Leonis** (Tul.) Rehm. On twigs and cone scales of *Picea*, Upper Brookside, July 4, 1931, (485); July 16, 1931, (1063); Salmon River, Sept. 7, 1931, (1479). These collections on *Picea* differ definitely from *P faginea* in the asci (130–200 × 20–23 μ), which in *P. Leonis* are more cylindric-clavate and long stalked and become more elongate with the spores arranged uniseriately, and the spores (23–31 × 9–14 μ), which are longer, have a thicker outer wall (2–2.5 μ), a granular rather than a biguttulate content, and are less strongly curved. *P. Leonis* is given as having a greenish hymenium, but Ell., N.A.F. 73, which was compared with *Stictis Leonis* Tul., and found to be the same, has a whitish hymenium as do Rel. Farl. 140 and Fl. Fayette Co., 1581 of *S. Leonis*. *Propolis Hillmanniana* W. Kirschst. (5, p. 203) may be this same species, although Kirschstein gives the spores of his species as being 40–50 × 10–14 μ.

Stictis radiata (L.) Pers. On bark of *Acer spicatum* and *A. saccharum*, Upper Brookside, July 15, 1929, (66); Salmon River, July 15, 1931, (1044). On decorticated *Betula*, Salmon River, July 15, 1931, (1036). These collections on bark differ from *S. mollis* in the snow white rather than greyish lips and the light coloured hymenium. The spores measure 190–250 × 1.5–2 μ.

Patellariaceae

Calicium abietinum Pers. On coniferous wood, Upper Brookside, July 11, 1931, det. J. H. Lowe, (490); July 22, 1929, (106).

Durella compressa (Pers.) Tul. On decorticated hardwood, Upper Brookside, June 29, 1931, (321), det. E. T. Butler.

— **lecideola** (Fr.) Rehm. On coniferous wood, Portapique Beach, Colchester Co., Aug. 7, 1933, (1618), det. E. T. Butler.

Hysteropatella minor (Cke.) Rehm. On decorticated *Betula*, Salmon River, July 15, 1931, (1077), det. E. T. Butler; North River Rd., July 7, 1931, (443). On decorticated Fagus, Upper Brookside, Aug. 11, 1931, (1662).

Karschia lignyota (Fr.) Sacc. On *Peniophora*, Upper Brookside, Aug. 3, 1929, (212); Salmon River, Aug. 18, 1931, (1355). The spores (7–9 × 3.5 μ) of these collections are somewhat smaller than given by Rehm for this species (9–12 × 4–5 μ).

Mycocalicium pallescens (Nyl.) Vain. On decorticated birch, Folleigh Lake, Aug 29, 1931, (1668), det. J. H. Lowe. Lowe (in litt.) states that this collection "lies between *Mycocalicium albonigrum* (Nyl.) Fink, ... *Chaenothecopsis pusiola* (Ach.) Vain. and *M. pallescens* (Nyl.) Vain. The last two differ essentially in having, or not having, respectively, an associated algal symbiont. I cannot find any algae in your material. Fink's description is too meagre to hazard a guess." Fink's material of *M. albonigrum*, in the Michigan Herbarium, is also meagre, consisting only of a few broken stalks. The Nova Scotia material is consequently referred to *M. pallescens*, which Lowe states is new to North America.

Patinella punctiformis Rehm. On dead wood, Economy Lake, Colchester Co., June 16, 1926, (P378). On decorticated fir, Portapique Beach, July 30, 1933, (1600).

Dermateaceae

Ascocalyx Abietis Naum. On *Abies balsamea*, North River Rd., Colchester Co., July 7, 1931, (416); Upper Brookside, June 19, 1933, (1524), det. J. W. Groves. The apothecia on No. 1524 are accompanied by the curious conidial stage, *Bothrodiscus pinicola* Shear, which has been connected with this species by Groves (4). These collections agree in all respects with Groves' descriptions.

Dermatea Ariae (Pers.) Tul. On *Sorbus americana*, Victoria Park, July 4, 1931, (402). Accompanied by the pycnidial stage, *Micropera Sorbi* (Fr.) Sacc. (*Sphaeronema pallidum* Pk.).

— **balsamea** (Pk.) Seav. On *Abies balsamea*, Upper Brookside, July 8, 1931, (439); July 18, 1931, (1082). On *Tsuga canadensis*, Victoria Park, Aug. 20, 1935, (1791).

All three collections are accompanied by the conidial stage, *Gelatinosporium abietinum* Pk.

— **Cerasi** (Pers.) Fr. On *Prunus* sp., Upper Brookside, Sept. 4, 1931, (1471), det. J. W. Groves. Accompanied by the conidial stage, *Micropera drupacearum* Lév.

— **molliuscula** (Schw.) Cash. On *Betula* sp., Upper Brookside, July 31, 1931, (1194); Aug. 12, 1931, (1287); Aug. 14, 1931, (1287a), det. J. W. Groves. This species fruits abundantly on down branches and cut brush of birch, appearing in the early summer as conidial stromata and in late summer as the dark brown to black apothecia. No. 1287 shows a conidial stage which is probably *Gelatinosporium fulvum* Pk. The elongate yellowish pulverulent stromata are erumpent through the periderm and contain one or many irregular cavities which open to the exterior. The conidiophores lining these cavities bear long, lunate-fusoid, hyaline, one-celled conidia measuring 45–60 × 3–3.5 μ. Collection No. 1194 shows similar stromata with cavities on the surface or more deeply seated. The finer hymenium of these cavities bears smaller conidia which are crescent shaped, hyaline one-celled, tapered at the ends and 7–12 × 1 μ. These conidia apparently represent a *beta* type of conidium for this species.

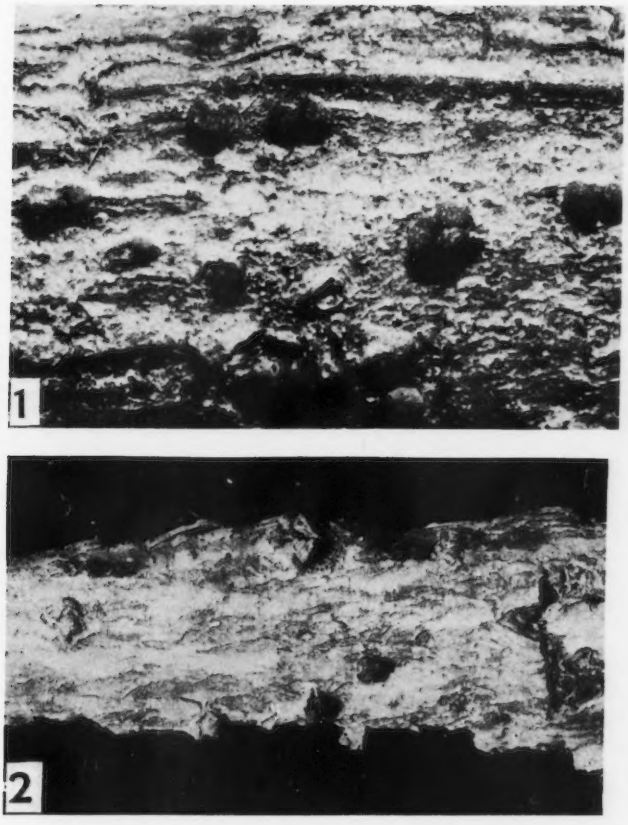


PLATE I, FIGS. 1 – 2. FIG. 1. *Surface view of apothecia of Pezicula Grovesii sp. nov.* ×12.
FIG. 2. *Surface view of apothecia of Tympanis acericola Groves. sp. nov.* ×4.

Godronia turbinata (Schw.) Farl. On *Diervilla Lonicera*, Victoria Park, July 6, 1931, (408); June 21, 1933, (1525); Portapique Beach, Aug. 3, 1933, (1613). Usually to be found on dead stems of this host.

— **urceolus** (Alb. & Schw.) Karst. var. **conferta** Hone. On *Prunus* sp., Salmon River, July 14, 1931, (1020), det. E. K. Cash. Miss Cash, in a letter, states "I have been referring Godronias on several host plants to *G. Urceolus*, as I have not been able to distinguish them from that species morphologically, but it is possible that the forms on different hosts are specifically distinct. . . . . This one on Prunus has shorter spores than typical *G. Urceolus* and apothecia usually in dense clusters, although this character is not apparent in your specimen. Both this Nova Scotia collection and one on Prunus from New Hampshire that I have examined, have a *Sirodiplospora* species associated with the *Godronia*, with two-celled fusoid spores borne on branched conidiophores."

Pezicula acericola (Pk.) Sacc. On *Acer saccharum*, Upper Brookside, July 15, 1929, (75); Salmon River, Sept. 7, 1931, (1060a). On *Acer spicatum*, Upper Brookside, July 26, 1929, (82); July 16, 1931, (1060). Common on living or dead bark of standing maple.

— **Alni** (Fck.) Rehm. On *Alnus* sp., Portapique Beach, Colchester Co., July 27, 1933, (1602), fide J. W. Groves.

Pezicula Grovesii sp. nov. (Figs. 1 and 3–5.)

Apothecia primum per rimas peridermatis minutas visibilia, discum obscurum vix visibile albidulum formantia, demum erumpentia, parva, diametro 0.2–0.4 mm., solitaria vel bina-quaterna aggregata, flavobrunnea vel pallide olivacea, aetate atriora, convexa, in stipite brevi, hyalino, parenchymatico, stromatico elevata; ascis crassis clavatis, basi angustatis, membrana paululum incrassata, 90–125 μ longis, 18–23 μ crassis; paraphysibus longis, filiformibus, simplicibus, diam. 1 μ, apice abrupte valideque expansis, ellipsoideis, brunneis, 8.5–14.0 μ longis, 5–7 μ latis, ascos superantibus et epithecium formantibus; sporis sursum biseriatis, demum uniseriatis, ellipsoideis vel inaequilateralibus vel paulum curvatis, hyalinis, granuliferis, 1-cellulis, 17–30 μ longis, 8–11 μ crassis. Specimen typicum in *Rhododendro canadensi* loco dicto "Upper Brookside," Nova Scotia, 8 Jul. 1931, in herbario L. E. Wehmeyer, n. 432.

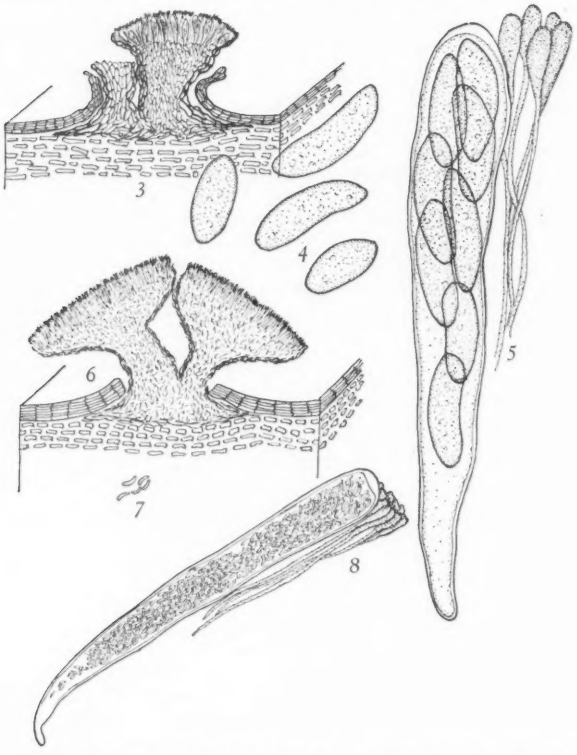
Apothecia (Fig. 1 & 3) preceded by a minute rupture of the periderm which exposes a barely visible whitish disc. Apothecia minute, 0.2–0.4 mm. in diameter, scattered singly or in small groups of two to four, yellowish-brown to pale olive-brown, drying darker, convex, erumpent on a short hyaline, parenchymatic, stromatic stalk, 200–300 μ in diameter. Asci (Fig. 5) stout clavate, with a tapered base and a slightly but evenly thickened wall, 90–125 × 18–23 μ. Paraphyses long filiform, simple, 1 μ in diameter, abruptly and strongly swollen above into ellipsoid, brown tips, 8.5–14 × 5–7 μ, projecting above the asci and forming an epithecium. Spores (Fig. 4) biseriate above, uniseriate below, ellipsoid to inaequilateral or slightly curved, hyaline, granular, one-celled, 17–30 × 8–11 μ. Type collection on *Rhododendron canadense*, Upper Brookside, Nova Scotia, July 8, 1931, in Herbarium L. E. Wehmeyer, No. 432.

The species name is chosen in commemoration of the excellent work done on the genus *Pezicula* by J. W. Groves. Material of this collection was sent to both J. W. Groves and Miss E. K. Cash, neither of whom were able to identify it as any known species. The writer, therefore has taken the responsibility of describing it as new.

The stroma arises on the surface of the bark cortex as a hyaline parenchyma with more or less parallel arranged cells measuring about 12–17.5 × 3.5–7 μ. This stroma ruptures the periderm and expands at the apex, appearing as a whitish disc. The hymenium soon forms over the entire convex surface and the walls of the surface cells of the stroma become darkened, giving the colour to the apothecium.

— **minuta** Pk. On *Viburnum Lentago*, Upper Brookside, July 13, 1931, (1002), det. J. W. Groves.

Sclerroderis Spiraeae Rehm. On *Spiraea tomentosa*, Oldham, Halifax Co., Sept. 5, 1929, (276). On *Spiraea* sp., Upper Brookside, July 28, 1931, (1171), det. E. K. Cash.



FIGS. 3 – 8. FIGS. 3 – 5. *Pezicula Grovesii sp. nov.* FIG. 3. *Vertical section of apothecium.* FIG. 4. *Ascospores.* FIG. 5. *Ascus and paraphyses.* FIGS. 6 – 8. *Tympanis acericola Groves. sp. nov.* FIG. 6. *Vertical section of apothecia.* FIG. 7. *Secondary ascospores.* FIG. 8. *Ascus with secondary ascospores and paraphyses.*

Tympanis acericola Groves sp. nov. (Figs. 2 and 6–8.)

Apothecia erumpentia, dispersa, caespitosa, raro solitaria, subsessilia, brevissime stipitata, orbicularia, vel undulata, 0.5–1.5 mm. lata, 0.5–1.0 mm. alta, nigra, dura, siccitate cornea vel cartilaginea, nondum siccata coriacea; hymenio plano vel convexo, margine primum elevato dein evanescenti; hypothecio plectenchymatico; ascis cylindrico-clavatis, breviter stipitatis, primum octosporis demum multisporis, (75)–85–110–(120) × (10)–12–15 μ; ascosporis primariis late ellipsoideis; 1-2-cellularis, hyalinis, uniseriatis, 4–5 × 2.5–3.5 μ; ascosporis secundariis minutissimis, hyalinis, allantoideis, 1.5–2.5 × 0.5 μ; paraphysibus hyalinis, filiformibus, septatis, furcatis, 1.5–2.5 μ diam., apice incrassatis longitudine usque as 3.4 μ conglutinatis, epithecium fuscum formantibus.

Hab.: in cortice *Aceris spicati* Lam.

Apothecia (Fig. 2 and 6) erumpent, scattered, caespitose in clusters of 2–8, occasionally single, subsessile, very short stipitate, circular, or undulate, 0.5–1.5 mm. in diameter, 0.5–1.0 mm. in height, black, hard, horny to cartilaginous in consistency, becoming tough-leathery when moist, hymenium plane to convex, at first with a raised margin which later disappears; tissue of the hypothecium plectenchymatous, hyaline to yellowish-brown, darker at the outside, forming a rind-like excipulum, the hyphae embedded in a matrix, apparently composed of the thickened, gelatinized walls. Asci (Fig. 8) cylindric-clavate, short stalked, at first eight-spored and very thick-walled, finally many-spored

and with much thinner walls, (75)–85–110–(120) × (10)–12–15 μ. Primary ascospores broadly ellipsoid, one- or two-celled, hyaline, uniseriate, 4.0–5.0 × 2.5–3.5 μ; secondary ascospores (Fig. 7) minute, hyaline, allantoid, 1.5–2.5 × 0.5 μ. Paraphyses hyaline, filiform, septate, much branched toward the tip, 1.5–2.5 μ in diameter, the ends clavate, swollen to 3–4 μ in diameter, and glued together to form a brownish epithecium.

Host: *Acer spicatum* Lam.

Type: Herbarium of J. W. Groves, 516, on *Acer spicatum*, Salmon River, Colchester Co., Nova Scotia, Sept. 7, 1931, leg. L. E. Wehmeyer (1476).

The only other *Tympanis* species heretofore described as occurring on *Acer* is *Tympanis acerina* Rehm on *Acer campestris*. In the description (12, p. 714) no mention is made of the primary ascospores, and the asci are given as slightly longer, 120–130 μ. In the Nova Scotia specimens only an occasional ascus reached 120 μ in length, but in other respects they agreed closely. In the genus *Tympanis*, the asci are apt to vary considerably in length, but the width is usually fairly constant, so that this difference may not be significant. This may be, therefore, the same fungus as that described by Rehm, but no specimens have been available for comparison. In any case, Rehm's name is invalid according to the International Rules, since the combination *Tympanis acerina* has already been used by Peck (7, p. 48) for the fungus now known as *Dermatea acerina* (Pk.) Rehm.

The above description and notes have been submitted by J. W. Groves, who kindly examined this material.

— **alnea** (Pers.) Fr. On *Alnus* spp., Green Oaks, Colchester Co., July 12, 1929, (176); Truro, July 12, 1929, (226); Victoria Park, Sept. 7, 1929, (284); Grande Anse, Richmond Co., Aug. 3, 1931, (1239), fide J. W. Groves. There are two distinct growth forms in these collections. Nos. 226 and 1239 have rather large apothecia, 0.5–1 mm. in diameter, very little incurved margin, are shiny black and occur in small groups of one to three with short stout stalks, often fused almost to the apex. In Nos. 176 and 284, on the other hand, the apothecia occur in large clusters of 5 to 20, are smaller (0.3–0.5 mm.), are definitely cup shaped with inrolled margins, and often show a whitish pulverulence. The stalks are also longer, narrower, and more distinct. The asci and spores appear to be the same in each group. Dr. Groves has kindly examined these collections and states (in litt.) that the pruinose character has been used to separate the species *T. conspersa*, but that he is convinced this is not a specific character. As to the striking differences in apothecial form he is more in doubt. Nos. 226 and 1239 probably represent *Tympanis hysterioides* Rehm. Groves states that the primary ascospores seem to be the same, however, and cultures he has succeeded in obtaining from both types of apothecia are identical.

— **fasciculata** Schw. On *Viburnum Lentago*, Upper Brookside, July 13, 1929, (272), fide J. W. Groves.

— **hysterioides** Rehm. See remarks under *T. alnea*. Nos. 226 and 1239 may be referred to this species.

— **Pinastri Tul.** On *Abies balsamea*, Moore's Lake, Halifax Co., July 6, 1929, (30 and 31); Upper Brookside, Aug. 20, 1931, (1658); Victoria Park, June 25, 1935, (1717), fide J. W. Groves. The collections of this species also show two distinct growth forms. Nos. 30 and 1658 have somewhat larger apothecia (0.6–1 mm.) which are flat-topped with a slightly raised and sometimes serrate margin. These apothecia are borne singly or two to three in a cluster and have short stalks. Nos. 31 and 1717 show larger clusters (3–10) of smaller apothecia (0.5–0.8 mm.) which are somewhat concave with a slight margin. The stalks of these apothecia are stout but more elongate (1–1.2 mm.). The writer was at first inclined to call Nos. 30 and 1658 *T. pithya* (Fr.) Karst. Groves (in litt.) believes, however, that they are merely growth forms and should be called *T. Pinastri*, inasmuch as cultures from both forms show identical colonies. A collection (No. 1659) of the

conidial stage, *Pleurophomella Pinastri* (Penz. & Sacc.) v. Hohn., has also been made on *Abies*.

— **pithya** (Fr.) Karst. See discussion under *T. pinastri*.

— **spermatiospora** Nyl. On *Populus*, Victoria Park, June 27, 1935, (1709).

Orbiliaceae

Orbilia inflatula Karst. On *Fagus grandifolia*, Victoria Park, Aug. 20, 1935, (1666), det. E. K. Cash.

— **leucostigma** Fr. var. **xanthostigma** (Fr.) Rehm. On *Polyporus betulinus*, Upper Brookside, July 30, 1929, (142). This may be *O. coccinella* var. *delicatula*, but it has the rosy colour when fresh and the smaller spores (3–3.5 × 1.5–2 μ) assigned to the above variety by Rehm (10, p. 455).

Orbilia luteorubella (Nyl.) Karst. On decayed stick, Upper Brookside, July, 1929, (45), det. E. K. Cash.

Mollisiaceae

Belonidium pruinosum (Jerd.) Rehm. On stromata of Hypoxylon, on *Fagus*, Victoria Park, June 26, 1935, (1702).

Mollisia benesuada (Tul.) Phill. On *Betula* sp., Victoria Park, Aug. 31, 1929, (266), det. E. K. Cash.

— **caespiticia** Karst. On stromata of *Nectria* and on bark, on *Acer* sp., Upper Brookside, July 17, 1935, (1746). The apothecia occur both on the stromata of the *Nectria* and also on the bark surface. When formed on the stromata, the young apothecia arise completely buried in the nectriaceous stroma.

— **cinerea** (Batsch) Karst. On decayed wood, Upper Brookside, July 14, 1929, (64); July 24, 1929, (97); June 27, 1931, (302); July 1, 1931, (352 and 358); July 7, 1931, (422).

— **melaleuca** (Fr.) Sacc. On decayed wood, North River Rd., Colchester Co., July 7, 1931, leg. A. H. Smith, (427), det. E. K. Cash.

— **stictella** Sacc. & Speg. On *Cornus alternifolia*, Upper Brookside, June 16, 1931, (1056). On *Spiraea* sp., Upper Brookside, July 13, 1931, (498). On *Acer spicatum*, Upper Brookside, July 16, 1931, (1056a). All determined by E. K. Cash.

— **uda** (Pers.) Gill. On blackened wood, Earltown Rd., Aug. 22, 1931, (1392), det. E. K. Cash.

Pseudopeziza Medicaginis (Lib.) Sacc. On alfalfa, East Cheboque, Yarmouth Co., July 30, 1931, leg. N. K. Andrews, det. A. R. Prince.

— **Artemisiae** (Lasch) Rehm. On *Solidago* sp., Truro, June 19, 1933, (1518). Both this species and the following variety are somewhat similar and common on dead *Solidago* stems. The perithecia of this species are smaller and darker at first and do not become so large, although they may become widely erumpent. They are practically sessile, have an exciple of dark brown, thick-walled parenchyma which appears black, and a margin which may be whitish but is not ciliate. The asci (33–38 × 3–4 μ) and spores (5–8.5 × 1–1.5 μ) are also smaller than those of the variety.

Pyrenopeziza Artemisiae var. **Solidaginis** Rehm. On *Solidago*, Upper Brookside, Aug. 1, 1929, (195); Truro, June 19, 1933, (1517). The apothecia of this variety are quite variable. At first they are almost spherical and dark coloured, with a central pore and a darker hymenium. Under favourable moisture conditions, they become widely erumpent and up to 1 mm. in diameter. They are often confluent, pale reddish-brown and have a light coloured hymenium. The stalk is also more pronounced than in the above species and the exciple is lighter in colour. The margin is often ciliate with free hyaline hyphae. The asci (40–50 × 4.5–6.5 μ) and spores (8–11.5 × 1.5–2.5 μ) are also larger.

Tapesia fusca Fckl. On decayed *Betula* sp., Salmon River, July 3, 1931, leg. A. H. Smith, (364), det. E. K. Cash.

— **melaleucoides** Rehm. On decorticated hardwood, Victoria Park, July 5, 1935, (1725).

Helotiaceae

Chlorosplenium aeruginascens (Nyl.) Karst. On decayed hardwood, New Glasgow Rd., July 25, 1931, (1155); Boularderie Island, Cape Breton Co., Aug. 4, 1931, (1155a). The asci of these collections are 35–48 × 3–5 μ and the spores 6–7 × 1.5–2 μ.

— **aeruginosum** (Oed.) de Not. On rotten wood, Folleigh Lake, Aug. 23, 1927, leg. A. R. Prince, (6039). The asci of this collection are 60 × 7 μ and the spores are 10.5–11.5 × 2–2.5 μ.

— **versiforme** (Pers.) de Not. On rotten wood, Princeport, Colchester Co., Aug. 26, 1927, leg. A. R. Prince, (6158); Earltown Rd., Aug. 21, 1931, leg. A. H. Smith, (1385). Rehm (10, p. 493) gives the spores of his *Coryne versiforme* as 9–14 × 3–4 μ, and the apothecia as 1–3 cm. in diameter. Phillips (8, p. 14) gives the spores of the same species as 8 × 4 μ and the apothecia as "5 lines broad." Collections made by the writer near Chelsea, Mich., agree well with Rehm, being 1–3 cm. in diameter and with spores 10–16 × 2–2.5 μ. Both of the Nova Scotia collections have smaller apothecia (2–8 mm.). No 1385 has spores 10–11.5 × 2–2.5(3) μ and agrees fairly well with Rehm's description. No. 6158 has spores 7–9 × 2.5–3(3.5) μ and fits Phillips' description. Either this species is widely variable, or there are two varieties with different spore sizes.

Dasyscypha Agassizii Berk. & Curt. On *Abies balsamea*, Moore's Lake, Halifax Co., July 6, 1929, (22); Upper Brookside, June 30, 1931, (330); July 13, 1931, (1662). Common on dead bark of fir.

— **occidentalis** Hahn & Ayers. On *Larix laricina*, Upper Brookside, July 8, 1931, (436). This is also close to *D. oblongispora* Hahn & Ayers, but seems to fit better the above species. The apothecia are deep orange, 0.5–1.5 mm. in diameter and thickly scattered. The asci are 96–106 × 8–9 μ and the spores 13–17.5 × 5–7 μ. The paraphyses are very slightly enlarged at the tip, if at all, and are 1.5–2.5 μ in diameter at the apex.

Helotium citrinum (Hedw.) Fr. On dead stem of *Alnus*, Upper Brookside, June 27, 1931, (308).

— **epiphyllum** (Pers.) Fr. On leaves of *Fagus*, Victoria Park, Aug. 17, 1931, leg. A. H. Smith, (1330). On leaves of *Fagus* and *Acer*, Earltown Rd., Aug. 22, 1931, (1330a).

Lachnella corticalis (Pers.) Fr. On bark of *Populus* sp., Salmon River, Aug. 13, 1931, (1299).

— **tricolor** (Sow.) Phill. var. **microspora** Kanouse. On decorticated scar areas of living beech, Upper Brookside, June 27, 1933, (1562), det. B. B. Kanouse.

Lachnum virgineum (Batsch) Karst. On decayed hardwood, Upper Brookside, July 4, 1931, (382). On *Acer spicatum*, Victoria Park, July 5, 1933, (1573). On beech bark, Upper Brookside, July 16, 1929, (41). On *Spiraea* stems, Upper Brookside, July 13, 1931, (497). Common.

Rutstroemia macrosporus (Pk.) Kanouse comb. nov. *Helotium macrosporum* Pk., Ann. Rept. N.Y. State Museum 26: 28. 1874. On decorticated wood, Upper Brookside, July 26, 1929, (96); Folleigh Lake, July 20, 1931, (1090); Mt. Thom, Aug. 15, 1931, leg. A. H. Smith, (1329), det. B. B. Kanouse.

This fungus is common on old decorticated stumps and exposed roots, blackening the substratum. Dr. Kanouse, who kindly examined this material, contributes the

following nomenclatorial revision of the species: "This species was published as *Rutstroemia nebulosa* (Cke.) Kauff. & Kanouse, (Pap. Mich. Acad. 9: 178. 1929). Since then the type of *Helotium macrosporum* Pk. has been examined and found to be identical. Seaver (Mycologia 26: 344. 1934), reinstated the pre-Friesian genus Calycina, and Peck's species, which he placed therein, became *Calycina macrospora* (Pk.) Seaver. Karsten (Myc. Fenn. 1: 12) published the genus Rutstroemia in 1871. According to the rules of nomenclature, this is a valid genus. In it belongs the fungus which has been reported under various names, some of which have been mentioned here. It becomes necessary to make the following new combination, *Rutstroemia macrospora* (Pk.) Kanouse."

Hysteriaceae

Graphis scripta (L.) Ach. On bark of *Abies*, Middle River, Victoria Co., Aug. 5, 1931, (1245).

— **scripta** var. **topographica** (Willd.) Zahlbr. On bark of *Betula*, Upper Brookside, July 16, 1929, (42); July 6, 1931, (405); Folleigh Lake, July 20, 1931, (1107), det. M. L. Lohman. Common on bark of various trees.

Hysterium pulicare (Pers.) Fr. On bark of *Betula*, Folleigh Lake, July 20, 1931, (1107); Salmon River, Sept. 2, 1931, (1459). On *Populus nigra* var. *italica*, Rosebank House, Baddeck, Victoria Co., Aug. 5, 1931, (1225), det. M. L. Lohman.

Hysterographium Mori (Schw.) Rehm. On *Fagus*, Upper Brookside, Aug. 11, 1931, (1276). On *Populus*, Salmon River, Aug. 1, 1931, (1197), det. M. L. Lohman.

Hypodermataceae

Hypoderma rufilabrum (Berk. & Curt.) Sacc. On twigs of *Acer spicatum*, Upper Brookside, July 16, 1931, (1061).

The copy of Rel. Farl. No. 127, in the University of Michigan Herbarium, shows spores (15.5–18 × 2.7 μ) somewhat shorter and more ellipsoid than those in this collection, which has elongate spores 21.5–30 × 3–4 μ, rounded at one end and tapered toward the other. A break in the protoplast, or a septum (?) appears at maturity. Tehon (in litt.) states that this collection is typical of *H. rufilabrum*, however.

Hypodermella nervata Darker. On leaves of *Abies balsamea*, Cheticamp River, Inverness Co., Aug. 6, 1931, (1255) det. M. L. Lohman. Parasitic, forming elongate apothecia along the midrib of dead leaves on living branches.

Lophodermium exaridum (Cke. & Pk.) Sacc. On leaves of *Kalmia angustifolia*, Portapique Beach, Colchester Co., July 22, 1935, (1667).

— **juniperinum** (Fr.) de Not. On *Juniperus communis*, North River Rd., Colchester Co., July 7, 1931, (415), det. M. L. Lohman.

— **melaleucum** (Fr.) de Not. On leaves of *Rhododendron canadense*, New Glasgow Rd., June 30, 1931, (333), det. M. L. Lohman.

— **nitens** Darker. On needles of *Pinus Strobus*, Victoria Park, June 21, 1933, (1663).

— **Piceae** (Fckl.) v. Höhn. On needles of *Picea canadensis*, Rifle Range, Truro, June 19, 1926, (P377), det. M. L. Lohman.

— **Pinastri** (Fr.) Chev. On needles of *Pinus Strobus*, Victoria Park, June 23, 1926, (P370), det. M. L. Lohman.

— **Tumidum** (Fr.) Rehm. On petioles of *Sorbus americana*, Victoria Park, June 23, 1933, (1537).

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THE RELATION BETWEEN FROST RESISTANCE AND THE PHYSICAL STATE OF PROTOPLASM

I. THE PROTOPLASM AS A WHOLE[1]

By J. Levitt[2] and D. Siminovitch[3]

Abstract

The protoplasm of hardy and non-hardy cells was compared with regard to displacement by centrifuging, rounding-up time on plasmolysis, deplasmolysis injury, rate of Brownian movement, resistance to and recovery from distortion by micromanipulation. The conclusions are: (i) When the cells are in the normally hydrated (i.e., turgid) condition there is either no difference in the consistency of hardy and non-hardy protoplasm, or else it is slightly less in the latter; (ii) when the cells are plasmolysed to the same degree (i.e., with a weaker plasmolyte in the case of the non-hardy) there is no difference in consistency; (iii) when the cells are in equilibrium with one and the same concentration of plasmolyte, the protoplasmal consistency of the non-hardy is greater than that of the hardy; (iv) the non-hardy are more susceptible to mechanical injury.

Evidence has recently been produced, on the one hand of an increased viscosity (2, 3) and on the other hand of a decreased viscosity (6) as a result of frost hardening. In the present paper an attempt will be made to clear up the discrepancies, both by a re-examination of the above results and by the addition of new evidence.

It should be recognized at the outset that a discussion of the physical state of protoplasm involves the use of somewhat controversial terminology. Since protoplasm is a non-Newtonian liquid the term viscosity cannot correctly be used. For this reason Seifriz (7) has suggested the term consistency. Both are the opposite of fluidity. In proportion, as the elastic properties (which may be present even in fluid protoplasm) increase, resistance to change of shape is more properly described as elasticity or rigidity rather than viscosity or consistency. But another physical property becomes important in "solid" protoplasm, namely, plasticity or ductility—the property of being deformed without fracture. The ability to prevent a break may also be due to elastic extensibility ("elasticity" in common parlance).

Since all gradations between the "fluid" and "solid" state exist, it is not always easy to decide which term should be adopted. This is especially true if the protoplasm is subjected to dehydration in steps, whereby its liquid properties progressively disappear.

In the following work, several methods of investigating the physical state of protoplasm have been used. The centrifuge, plasmolytic, and Brownian movement methods presumably measure consistency, since all depend on the fluid characteristics of protoplasm. Resistance of cytoplasmic strands to

[1] *Manuscript received June 17, 1940.*
Contribution from the Department of Botany, McGill University, Montreal, Que.
[2] *Sessional lecturer in Botany.*
[3] *Assistant in Botany.*

tear, and of the protoplast to rupture on deplasmolysis, must be more in the nature of ductility or plasticity. Evidence of this is found in the fact that broken strands remain flagella-like, instead of rounding up as would occur in the presence of surface tension forces. Micromanipulation is capable of yielding information about both the fluid and solid characteristics of protoplasm.

Though sound terminology is essential for clarity, from the point of view of frost resistance, it is relatively unimportant whether, for instance, deplasmolysis injury is due to high consistency or low plasticity (or ductility). The important facts are that the condition is physical and that it varies in a definite way with change in temperature and hardiness.

The Centrifuge Method

Kessler (2) demonstrated that the plastids of hardy plants are less readily displaced than those of non-hardy plants. Recognizing the possibility of an altered specific gravity due to the presence or absence of starch in the plastids, he performed the following control experiment. Non-resistant plants were placed in the dark until all the starch had disappeared (six days). The plastids in these starch-free plants proved more easily displaced than those in the untreated. Obviously, this could not be due to an increased specific gravity of the plastids, and Kessler did not attempt to interpret it so (as stated by Scarth and Levitt (6)). In the later paper, Kessler and Ruhland (3) ascribe the result to a change in the protoplasm. In any case, it was impossible to decide from these results whether the difference in starch content was responsible for the more ready plastid displacement in non-resistant than in frost-resistant plants.

In the case of *Hedera*, on the other hand, since both resistant and non-resistant forms contained starch, Kessler and Ruhland assert that this did not influence the displacement. Yet even in this case, unless *Hedera* is an exception, the quantity of starch in the plastids must have been greater in the unhardened plants. Geffken (1) has, in fact, found that *Hedera* shows the usual carbohydrate changes associated with hardiness.

In the case of *Catalpa*, plastid displacement was too rapid to allow the recognition of a difference in rate. On the other hand, when the starch was allowed to disappear (in the dark), a very high speed was necessary to obtain any displacement at all. Even then, the movements proved to be centrifugal in the resistant, and centripetal in the non-resistant. Thus, even in the absence of starch, hardy and non-hardy cells apparently showed differences in the relative specific gravities of plastids and protoplasm.

Kessler and Ruhland produced further evidence in an attempt to prove the validity of the centrifuge results. Narcosis rapidly reduced frost resistance and allowed a more ready displacement of the plastids. In this case, they state, it must have been the protoplasm that was altered, since both sets of plants were without starch and it is therefore due to a difference in viscosity.

Yet even if narcotized plants are less resistant, and at the same time less viscous than the unnarcotized, this fails to prove that the same holds true for plants differing naturally in frost resistance—especially since the lower "frost-resistance" of narcotized plants may be only apparent. On ice-formation, the concentration of narcotic in the protoplasm increases, and death may be due to this rather than to a reduction in true frost resistance.

Since really hardy cells contain starchless chloroplasts, the centrifuge method is applicable only to those that are starchless in the non-hardy condition as well. The cortical cells of *Catalpa* and *Cornus* when dehardened in the laboratory during winter fulfil this condition. Yet the difference in frost resistance in the two states is enormous. Cortical sections from the dehardened twigs were completely killed by freezing at about $-7°$ C.; those from hardy twigs were uninjured by $-30°$ C. (the lowest temperature tried). In both cases the sections were inoculated with ice to insure freezing.

There is one important cellular difference between *Catalpa* and *Cornus*. In the former, the cortical protoplasts all consist of a layer of cytoplasm surrounding a single large vacuole which stains intensely with neutral red. In the latter plant, only half the cortical cells are of this type. The others have no single large vacuole—neutral red either fails to stain them or at most causes a faint brownish red coloration. These will be called "avacuolate" as opposed to the other "vacuolate" cells. Both types are shown in Plate I, A.

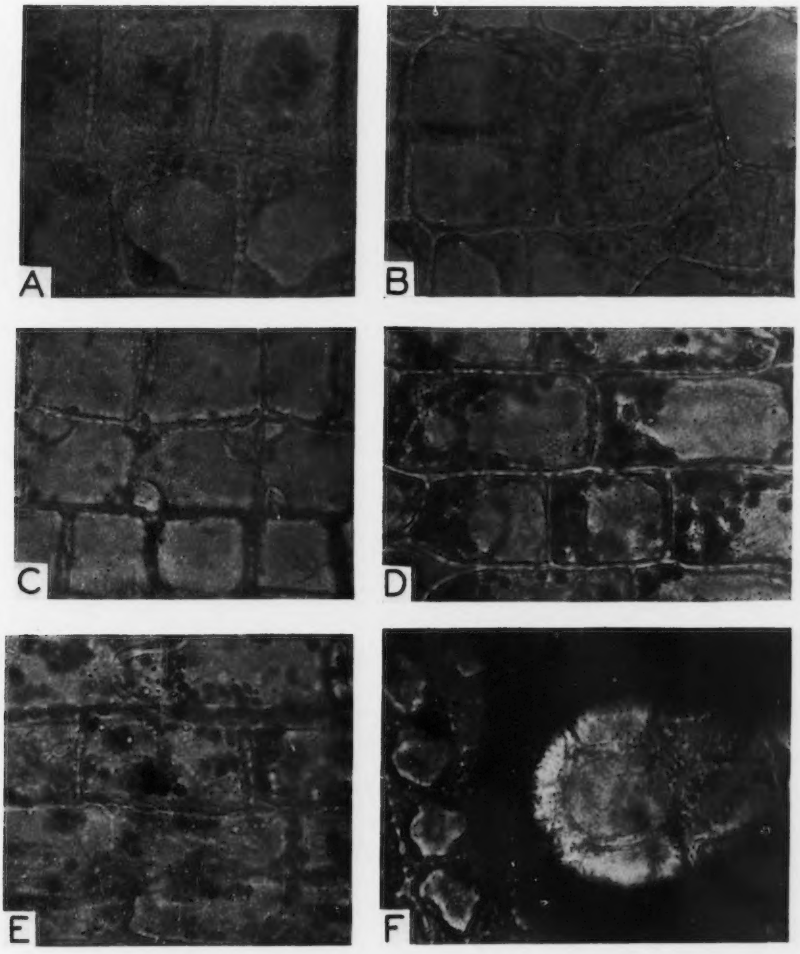
Tangential sections were placed lengthwise in small tubes of water which were inserted in the containers of a hand centrifuge with a radius of either $2\frac{1}{2}$ or 7 in. They were then centrifuged for $\frac{1}{2}$ to 10 min. at 1800 to 4200 r.p.m. and immediately examined. From the known radius, r, and r.p.m., the relative centrifugal force was calculated, using the formula:

$$F = \left(\frac{\text{r.p.m.}}{300}\right)^2 \times r$$

The number 300 was chosen arbitrarily to reduce the otherwise unwieldy figure.

Table I shows that the hardy "avacuolate" cells always permitted readier displacement of their chloroplasts than did the dehardened cells. The difference is clearly revealed in Plate I, B – E. This can be taken as evidence of lower protoplasmal consistency in the hardy than in the dehardened cells only if in both cases (i) the relative specific gravities of the chloroplasts and cytoplasm are the same; (ii) the chloroplast size is the same; (iii) the thickness of the cytoplasm layer is sufficient to permit passage of the chloroplasts. The absence of starch in both cases favours but does not guarantee the fulfilment of all three of these conditions.

In the "vacuolate" cells, however, it is not merely a matter of chloroplast movement. The protoplasm and the vacuole are displaced relative to each other (Plate I, B and D). (It may be noted that hardy *Catalpa* cells showed centripetal as opposed to the centrifugal displacement recorded by Kessler and Ruhland.) Here again relative specific gravities may conceivably be



A. Cortical cells of hardy Cornus. Upper row "avacuolate", lower row "vacuolate." B. Cortical cells of hardy Cornus after centrifuging for 2 mins. at 1800 r.p.m. Right centrifugal. C. Cortical cells of dehardened Cornus after centrifuging for 2 mins. at 1800 r.p.m. Right centrifugal. D. Cortical cells of hardy Catalpa after centrifuging for 2 mins. at 1800 r.p.m. Right centrifugal. E. Cortical cells of dehardened Catalpa after centrifuging for 2 mins. at 1800 r.p.m. Right centrifugal. F. The application of pressure on cortical cells of hardy Cornus by means of a blunt microneedle.

TABLE I

THE EFFECT OF CENTRIFUGING ON CORTICAL CELLS OF HARDY AND DEHARDENED *Cornus* AND *Catalpa* TWIGS

F = *centrifugal* displacement of *chloroplasts*. *P* = *centripetal* displacement of *protoplasm* as a whole

F (relative centrifugal force)	Time centrifuged, min.	Hardy			Dehardened		
		Cornus		*Catalpa*	*Cornus*		*Catalpa*
		"Avacuolate" cells	"Vacuolate" cells		"Avacuolate" cells	"Vacuolate" cells	
252	2	*F*	*P*	*P*	*O*	*O*	*O*
252	5	*F*	*P*	*P*	*O*	*O*	*O*
252	10	*F*	*P*	*P*	*F*	*P*	*P* (Slight)
360	1	*F*	*P*	*P*	*O*	*O*	*O*
360	4	*F*	*P*	*P*	*O*	*O*	*O*
360	7	*F*	*P*	*P*	*F* (Partial)	*P* (Partial)	*P* (Partial)
490	½	*F*	*P*	*P*	*O*	*O*	*O*
490	10	*F*	*P*	*P*	*F*	*P*	*P*

responsible for the difference between hardy and dehardened cells. In any case, the protoplasm of the hardy cells (and they are *very* hardy) is evidently quite fluid or it would not be so readily displaced in all cases. Consequently these results at least oppose the contention that frost resistance is correlated with high protoplasmal consistency. They may even be interpreted as indicating a greater consistency in dehardened than in hardy cells.

But there are reasons for expecting a difference in the relative specific gravities of plastids, protoplasm, and cell sap in frost-resistant and non-resistant cells. The specific gravities of both protoplasm and cell sap are bound to change with increased frost resistance, owing to the increased hydration of the former (3, 6) and the increased osmotic concentration of the latter. The plastids themselves are modified during winter, apparently independently of the carbohydrate change, for Kraus (4) was able to reverse this change by a one- to two-day exposure to warm temperatures.

Owing to these differences, the centrifuge method is not a reliable test of relative protoplasmal consistency in frost-resistant and non-resistant plants.

The Plasmolytic Method

In the absence of complicating factors, the centrifuge method would reveal the relative consistency of protoplasm in a fully turgid cell. The plasmolytic method, on the other hand, involves a partial dehydration of the protoplasm. It should, therefore, be recognized that even under ideal conditions the two may not give identical results (unless plasmolysed cells are centrifuged), for it is quite conceivable that the dehydration may alter the protoplasmic consistency to a greater degree in one case than in another.

In contrast to the results of Scarth and Levitt (6), Kessler (2) and Kessler and Ruhland (3) recorded a more rapid rounding up of non-resistant than of resistant cells. The latter explain the results of Scarth and Levitt as due to (i) the more rapid evaporation from the less concentrated solution (containing the non-resistant cells) when the evaporation method was used, and (ii) to the Ca^{++} effect. A theoretical consideration of these suggestions is unnecessary, since this was not the only method that yielded these results. At the time when protoplasmic measurements were being made (Scarth and Levitt) it was always more difficult and time-consuming to obtain dehardened cells in the rounded condition, at least in the higher concentrations. Dextrose was the plasmolyte, and no evaporation was involved. However, the sections had previously been vitally stained for half an hour in a weak calcium chloride solution containing neutral red, and for this reason the experiments required repetition. Results with hardened and unhardened cabbage also agree with these, though the differences were relatively small.

The complete absence of electrolytes should eliminate the complication of adhesion (5), provided that there are no naturally occurring polyvalent cations in the cell wall. But plasmolysis shape alone is independable as an indication of relative consistency. Thus both hardy and dehardened *Catalpa* cells, when plasmolysed moderately in dextrose solutions are concave, both hardy and dehardened *Cornus* convex. On the other hand, *Catalpa* cells that have been dehardened for over a month have a very low osmotic concentration and show perfect convex plasmolysis. Hardy *Hydrangea* cells all tend to plasmolyse convexly, but the dehardened cells mostly plasmolyse concavely.

Determinations of rounding-up time, however, should yield some information as to the relative consistencies, provided that in both cases the protoplasts are deformed to the same degree. Since it is not possible to exert much control on the protoplast shape without affecting its consistency, the usual procedure is merely to plasmolyze to the same degree.

Preliminary experiments were performed to test Kessler and Ruhland's criticism. Sections of both hardy and dehardened *Catalpa* were put in hypotonic calcium chloride for some time (one or more hours), after which they were transferred to hypertonic dextrose. Control sections were cut directly into another vial containing the same concentration of dextrose. There was no appreciable difference in the times required for rounding-up, whether or not the sections had been pretreated with the calcium chloride. And this held true both for the hardy and dehardened *Catalpa*. This confirms the value of the results mentioned above.

Comparison of hardy and dehardened cells was made by sectioning the twigs and immediately placing the sections into the plasmolyte (dextrose). *Catalpa* cells are good for this test since both hardy and dehardened plasmolyse with about the same degree of irregularity.

It is seen from Table II that the dehardened were able to round up in two thirds of the time required for the hardy when both were plasmolysed to about

TABLE II

ROUNDING-UP TIME OF HARDY AND DEHARDENED CORTICAL CELLS OF *Catalpa*

Dextrose conc., *M*	Rounding-up time, hr.	Degree of plasmolysis. (Average of 10 cells)
(a) Dehardened (controls killed by −5° C.)		
0.4	1¾	
.5	2	0.77
.6	2⅓	
.7	3	
.8	5	.63
.9	6	.53
1.0	10	.48
(b) Hardy (uninjured by −15 to −20° C.)		
0.9	3	0.72
1.1	4½	.69
1.3	7	.67

three-quarters of their normal value. But the figures reveal that slightly greater volume changes cause disproportionately great increases in time. This is undoubtedly due to the effect of the increased plasmolyte concentration on protoplasmic consistency. If, then, we compare the rounding-up time in the same concentrations of plasmolyte, (i.e., under the same dehydrating conditions) the hardy are found to round up two or more times as fast.

This is in agreement with the results of Scarth and Levitt. When the evaporation method was used, the plasmolyte became highly concentrated and to about the same degree in both cases. (In spite of Kessler and Ruhland's objection, the osmotic pressure of a solution has relatively little effect on its rate of evaporation.) It also confirms the results of Kessler and Ruhland who observed a more rapid rounding up in the case of dehardened cells plasmolysed to two-thirds of their normal volume than in the case of hardy cells plasmolysed to the same degree.

The results do indicate a lower consistency in dehardened than in hardened cells when compared at their isotonic points—as is done when the centrifuge method is used. But this seems only true when the dehardened cells possess a strikingly lower osmotic pressure than the hardened cells. Thus it was shown by Scarth and Levitt that hardy cabbage cells round up more rapidly than do dehardened. These experiments have been repeated and confirmed.

Further evidence of this was obtained with *Catalpa*. A twig, dehardened in the laboratory for a month, had grown two shoots more than an inch long. The osmotic pressure of the cortical cells had dropped to the very low value of 5 to 6 atm. These cells rounded up almost immediately in hypertonic

dextrose solutions. Obviously the protoplasm in equilibrium with this low cell-sap concentration had so high a content of "free" water that its consistency was practically that of water itself. Rounding-up occurred immediately, before the protoplasmic dehydration was sufficient to increase its consistency markedly.

It is apparent from this that the method of rounding-up time is inapplicable when the cells being considered differ greatly in osmotic concentration and non-solvent space. A further complication is in this case introduced. The method assumes that the consistency of the cell sap is negligible in comparison with that of the protoplasm. But in the case of hardy cells it seems certain that the sap is sufficiently concentrated and contains sufficient colloidal matter to endow it with a considerable degree of viscosity. Furthermore, if two cells possess the same protoplasmic consistency, the one with the thinner layer will round up the more rapidly. Hardy cells have thicker protoplasm layers than non-hardy ones, at least in some cases. Finally, it should be remembered that plasmolysis tests involving periods of several hours may not reveal true consistency, owing to changes that may occur during this time. (That a change does occur will be shown in a later section of this paper.)

For these reasons, it is desirable to employ other methods as a check on the results already described.

Deplasmolysis Injury

Scarth and Levitt (6) showed that hardy cells survive deplasmolysis after plasmolysis in strong solutions, while unhardened cells are uninjured only if the plasmolyte is much weaker. They attributed this "deplasmolysis resistance" of hardy cells to a lower protoplasmic viscosity, assuming that the bursting of the cell during deplasmolysis is due to the high consistency either of the protoplasm as a whole or of its membrane. Evidence of this was the fact that the injury increased when the temperature was lowered during the deplasmolysis. Kessler and Ruhland (3) suggest that plasticity rather than viscosity is the true explanation. Since all the above experiments employed a balanced solution of sodium chloride and calcium chloride (9 : 1), it would seem advisable to test the effect of sugars, in order to eliminate any specific ion effect that may differ in the case of hardy and non-hardy cells.

Using dextrose, it was found that unhardened cabbage cells were completely killed on deplasmolysis from a 2.0 *M* solution, and 50% killed from 1.5 *M*. Hardened cells showed 50% killing only when the 2.0 *M* concentration was used. *Catalpa* gave similar results. Naturally hardened cortical cells were killed by deplasmolysis when 4 *M* sucrose was the plasmolyte; dehardened cells suffered the same injury after plasmolysis in 2 *M* sucrose. But the injury in the 4 *M* sucrose was probably not deplasmolysis injury. The high concentration of sucrose tends to cause vacuole contraction rather than plasmolysis, and this abnormal reaction is in itself injurious. And since the tests were made in December, the cells had not yet reached their maximum degree of hardiness. When tested later in the winter, using solutes that do

not cause vacuole contraction (dextrose or a balanced solution), no deplasmolysis injury was obtainable with the highest concentrations (e.g., 6 *M* dextrose), even though these possessed greater osmotic values than the 4 *M* sucrose.

In the case of cabbage, the increase in concentration required to produce the same injury in hardy as in non-hardy cells was probably just sufficient to counterbalance the cell sap differences. In other words, the cell contraction in the plasmolytes was about the same in the two cases. Deplasmolysis injury, however, must depend not only on the degree of expansion the cell is exposed to, but also on the physical state of the protoplasm. It would, therefore, seem that the hardy cell had about the same protoplasmic consistency in the more strongly dehydrating solution as the non-hardy one in the less strongly dehydrating solution. This difference between hardy and non-hardy cells is obviously less than that previously obtained. The explanation of the apparent discrepancy will appear in Part II of this series.

Brownian Movement

Scarth and Levitt were unable to detect any difference in the rate of movement of particles in hardened and unhardened cabbage. In hardy *Catalpa* no movement was seen. A re-examination of the latter has revealed the reason for this. The hardy *Catalpa* cell (as seen from above) is surrounded by a broad ring of protoplasm. Observation of this peripheral zone involves a view of a thick layer filled with plastids and various particles. It would be very difficult to see any movement here. If, however, the microscope is focused on the upper surface of the protoplast, a relatively thin and transparent layer is viewed and the number of particles seen is much less. These are clearly in Brownian movement, and even streaming.

Cortical cells of both hardy and dehardened *Catalpa* and *Hydrangea* were observed. The hardy twigs were taken indoors in April and placed in a cold chamber at about 0° C. At about the time that observations were made, some twigs were frozen at temperatures down to −17° C. without suffering the slightest injury. They all sprouted within about a week of this. (To insure freezing, the cut ends of the twigs were standing in a layer of distilled water.)

The dehardened twigs were collected in April, and kept in water in the laboratory for two or more weeks. When tested, they all had green young sprouts. Twigs frozen in the cold chamber at −5° C. proved to be dead several days later.

Instead of attempting to distinguish a difference in rate of movement, which is very difficult and highly subjective, the sections were placed in solutions of different concentrations, and the concentration capable of stopping the movement was determined. A concentration of 0.50 to 0.75 *M* dextrose was sufficient in the case of dehardened *Catalpa* and *Hydrangea*. Hardy *Hydrangea* required 1.0 to 1.5 *M*, hardy *Catalpa* 1.5 to 2.0 *M*.

Here again, the hardy cells proved to have a lower consistency than the dehardened when in equilibrium with the same dehydrating forces.

Micromanipulation

This method investigates the relative resistances to distortion offered by protoplasm in hardy and dehardened cells. Tangential cortical sections of *Catalpa* or *Cornus* twigs were mounted in a hanging drop of a balanced solution (sodium chloride and calcium chloride in the molar ratio of 9 : 1). One micro-needle held the section rigidly in place, while the other was left free for manipulation.

The thin pits in the otherwise thick walls offered an easy locus for the penetration of the needle, but only when the cells were plasmolysed. Plasmolysis was also necessary for observation of the effects of manipulation. Frequent renewal of the solution compensated for evaporation during manipulation.

Three main methods were used for investigating the relative consistencies of hardy and dehardened cortical cells of *Catalpa* (Methods 1 and 2) and *Cornus* (Method 3).

(1) The protoplasts were manipulated in two ways: a furrow was made in the protoplasm and recovery after removal of the needle was noted; or pressure was exerted on the wall, causing distortion of the protoplast, the release of pressure permitting reversion to its original shape. In both cases relative fluidity could be estimated by the ease (i.e., the speed) with which the protoplasm was able to change its shape.

In all cases an increase in consistency occurred with increased dehydration. In a balanced solution with an osmotic concentration of about 50 atm., the protoplasm of hardy cells flowed easily and responded quickly to pressure, resembling a thin, sticky glue. The protoplasm of dehardened cells behaved like a viscous, flowing plastic. But the osmotic concentration of hardy cells (20 atm.) is about double that of dehardened ones. A comparison was therefore made using $2\frac{1}{2} \times$ isotonic solutions for each (i.e., with osmotic concentrations of 50 and 25 atm. respectively). In this case there was no apparent difference in consistency between the protoplasm of hardy and dehardened cells. Similarly, a solution with an osmotic concentration of 100 atm. for hardy cells and one with an osmotic concentration of 50 atm. for dehardened cells, failed to reveal a difference. When the concentrations were again doubled (200 and 100 atm. respectively), the consistency in both cases was such as to allow almost permanent molding into any shape.

Manipulation caused coagulation of the protoplasm and puncturing of the vacuole much more frequently in dehardened cells than in hardy ones.

(2) In the second method, a pit in the wall was punctured and the protoplast squeezed out of the hole by pressure on another part of the wall. The shape of the extruded mass was taken as an indication of relative consistency. By first staining with neutral red, it was possible to observe that the protoplasm and not the vacuole was extruded in all the cases discussed. The results are shown in Table III and Fig. 1.

TABLE III

SHAPE OF PROTOPLASMIC EXTRUSIONS FROM HARDY AND DEHARDENED CORTICAL CELLS OF *Catalpa*

	Osmotic concentration (atm.) of balanced solutions		
	25	50	100
Hardy	Spherical	Pear-shaped	Narrow tongue with irregular thickenings
Dehardened	Spherical→pear-shaped	Narrow tongue with irregular thickenings	Could not be squeezed out

If deplasmolysis was permitted, a spherical extrusion occurred from both hardy and dehardened cells. In the dehardened, however, only a slight expansion occurred before the extruded mass burst. In the hardy, the extruded mass burst only after expanding to fully the size of the cell itself.

As in the first method, the hardy protoplasm showed a much greater tolerance toward micromanipulation than did the dehardened. It could be extruded and retracted again and again, and even torn away and another mass of protoplasm extruded without any coagulation. The dehardened protoplasm on the other hand, usually coagulated at the first extrusion. Consequently only a minority of the cells could be used—cells with protoplasm having an apparently reduced viscosity as judged by the increased vacuolation.

(3) In the third method, a more or less uniform pressure was applied to the cells by means of the well-rounded end of a blunt micro-needle (Plate I, F). Both the ease of deformation and the speed of readjustment were noted.

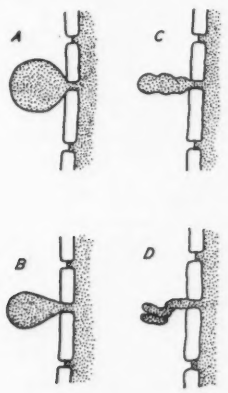


FIG. 1. *Diagrammatic representation of shapes of protoplasm extrusions from a punctured pit. A = spherical, B = pear-shaped, C and D = narrow tongue with irregular thickening.*

In solutions of similar relative osmotic concentrations (25 atm. for the dehardened, 50 atm. for the hardy) consistency was about the same in the dehardened and hardy cells. In solutions with 4 × these concentrations (100 atm. and 200 atm. respectively), the protoplasts retained their flattened configuration after the pressure was released. In solutions with the intermediate concentration (100 atm.) the hardy protoplasts were still able to resume their original shape after release of pressure, though not as quickly as in a solution of 50 atm. concentration. In solutions with osmotic concentrations of 400 atm. and greater, the protoplasm was apparently a solid, for it fractured as a result of pressure, allowing escape of the vacuole contents.

Hardy protoplasts expanded readily under such pressures, sprang back to their original shape on release of the pressure, and in most cases were even capable of deplasmolysis. They were injured only if the pressure was great enough to cause a sudden extrusion through a burst pit. Dehardened cells, however, were killed long before expansion to the degree permitted by hardy cells. Sometimes the dehardened protoplast "exploded", as though it had reached the limit of its extensibility.

All these micromanipulation results indicate that:

(1) When the cells are in the unplasmolysed condition there is no appreciable difference between the protoplasmal consistency of hardy and dehardened cells.

(2) When the cells are in equilibrium with hypertonic solutions bearing the same relation to the isotonic value of the respective cells (about twice as great in hardy as in dehardened *Catalpa*), there is also little difference between hardy and dehardened.

(3) When the cells are in equilibrium with one and the same concentration of plasmolyte, the protoplasmal consistency of the dehardened is greater than that of the hardy. The difference increases with the concentration of the solution.

(4) When the hardy and dehardened cells are compared under any one of the above three sets of conditions, the dehardened are more susceptible to mechanical injury.

But how do these conclusions agree with those arrived at by means of the other three methods?

(1) Results with the centrifuge method prove that hardy protoplasm is fluid in turgid cells. They might also be interpreted as indicating a higher consistency in dehardened than in hardy cells, were it not for the complication due to differences in relative specific gravities of protoplasm, plastids, and cell sap. Results with the rounding-up time technique indicate the opposite—a *lower* consistency in dehardened than in hardy cells, but only when the cell sap concentration of the dehardened is much lower than that of the hardy, and the protoplasm is therefore in equilibrium with a much lower dehydrating force. It is quite possible that the micromanipulation method may be incapable of detecting differences in consistency between two systems in this

highly fluid state. The results obtained with the Brownian movement method throw no light on this point.

(2) Results obtained with the plasmolysis-time method indicate a higher consistency in the hardy cells. But this evidence cannot be considered as conclusive since the rounding-up time (five to seven hours) was long enough for the occurrence of protoplasmic changes (see later section). Results with the deplasmolysis method, however, agree with those obtained by micromanipulation.

(3) The centrifuge method does not deal with this point. *All four remaining methods agree.*

(4) This point does not throw any light on the consistency of the protoplasm as a whole, but as will be shown in Part II of this series, depends on the rigidity of the ectoplasmic layer.

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VARIABILITY IN ASSOCIATION EFFECTS OF OTHER SOIL FUNGI ON THE VIRULENCE OF *HELMINTHOSPORIUM SATIVUM* ON WHEAT SEEDLINGS[1]

By G. B. Sanford and M. W. Cormack[2]

Abstract

Random isolates of *Penicillium*, *Actinomyces*, and certain miscellaneous soil-inhabiting fungi were tested in steam sterilized soil, under pure culture conditions, for their association effects on the virulence of *Helminthosporium sativum* P. K. and B. on wheat seedlings. Certain isolates of the first two genera mentioned exerted a marked degree of suppression, some had no effect, while others increased the virulence. Similarly, these effects varied widely within certain species of *Penicillium*. This preliminary study indicates that the random isolates of many genera and species of fungi may differ widely in ability to affect the virulence of certain plant pathogens.

The ability of various bacteria and fungi to reduce the virulence of certain plant pathogens in soil culture has been established. However, no one seems to have determined the diversity in this connection that may exist within a given genus or species.

Following is a preliminary report of experiments, carried out under pure culture conditions, to demonstrate the range of antagonism exhibited by 24 isolates of *Penicillium* spp., eight isolates of *Actinomyces* spp., and several miscellaneous fungi, to the virulence of *Helminthosporium sativum* P. K. and B. on wheat seedlings.

Methods

In 1933, dilutions from composite samples of soil from four wheat fields near Edmonton, Alberta, were plated out in suitable media. The numerous isolates of fungi secured were differentiated by means of cultural and microscopic studies, and types that occurred most frequently in each soil were selected for the present study. Single spore cultures were used in all experiments. Dr. G. R. Bisby, Imperial Mycological Institute, Kew, England, and Dr. J. E. Machacek, Dominion Laboratory of Plant Pathology, Winnipeg, kindly identified a number of the isolates listed in Table I.

A technique was developed for growing wheat seedlings aseptically in wide-mouthed, one-litre, Erlenmeyer flasks with only the pathogen and one associate present. All flasks contained 300 gm. of a 3 : 1 soil-sand mixture of uniform moisture content. The flasks, each stoppered with a special cotton plug containing a circular wire frame and a wire stirring rod, were autoclaved twice for a period of four hours on successive days at 15 lb. pressure. As this special stopper was not removed until the end of the experiment, the chances

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Contribution No. 629, Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] *Plant Pathologist-in-Charge and Assistant Plant Pathologist, respectively, Dominion Laboratory of Plant Pathology, Edmonton, Alta.* *Read at meetings of Canadian Phytopathological Society, Winnipeg, June, 1940.*

for contamination were practically eliminated. All materials were introduced aseptically through a small hole in the centre of the cotton plug. This hole, which was supported by the wire frame mentioned, was plugged with cotton.

Standardized spore suspensions consisting of the pathogen only, of the pathogen with the particular associate to be tested, and of each of the associates only, were prepared and 50 cc. of the given spore suspension was pipetted into each flask as required. Four flasks were used for each treatment.

Marquis wheat seed was soaked for three hours in water and then placed in a 1 : 1,000 solution of mercuric chloride for seven minutes. This method of seed disinfection proved most satisfactory for producing sterile seedlings. Following the application of the various spore suspensions indicated, 20 of these treated seeds were put into each flask, then distributed and slightly buried with the stirring rod. A layer of fairly dry, sterile sand was then spread over the planted surface to a depth of one-half inch. This sand prevented the mycelium of the pathogen from growing on the surface and attacking the seedlings above the soil line.

The water content of the soil, when the seeds were planted, was ideal for good plant growth, and water was not added during the incubation period. Plating tests showed that practically all flasks remained uncontaminated at the end of the experiment. All contaminated ones were discarded. Final notes on germination, height of plants, and infection were taken at the end of three weeks. The three groups of fungi mentioned were tested at different times.

Results

Penicillium spp.

Penicillium spp. were isolated much more frequently than any other fungus. Therefore, a relatively large number of these random isolates were examined for association effects on *H. sativum*. The effect of each isolate on wheat seedlings with the pathogen absent was also determined. When the isolates were associated with *H. sativum* the virulence of this pathogen, as indicated by the infection ratings in Table I, ranged from 16 to 97%, in comparison to 73% for *H. sativum* alone. *P. simplicissimum* (Oud.) Thom, and *P. fuscus* Sopf., depressed this rating by 57 and 42%, respectively. One isolate of *P. chrysogenum* Thom, depressed virulence by 39%, while another increased it by 20%. One isolate of *P. expansum* Thom, depressed virulence 31%, while two others indicated very little, if any, effect. An isolate of *P. glabrum* (Wehmer) Westling depressed the virulence by 24%, and two isolates of *P. terrestre* Jensen by 18 and 11%, respectively. *P. Trzebinskii* Zaleski, had no effect and *P. rugulosum* Thom increased it by 22%. The four isolates of *P. viridicatum* Westling indicated wide diversity in its effect upon virulence of the pathogen; two reduced it by 22 and 17%, respectively, one had no definite effect, while the fourth increased virulence by 11%. One isolate of *P. chloroleucon* Biourge depressed the virulence by 17%, and another had no effect. Two isolates of *P. cyclopium* Westling had no definite effect. Of the

TABLE I

THE EFFECT OF RANDOM ISOLATES OF CERTAIN SOIL FUNGI ON THE VIRULENCE OF *Helminthosporium sativum* ON WHEAT SEEDLINGS

Isolate	Dead plants	Av. ht., cm.	Av. rt.,[1] %
Penicillium			
P. simplicissimum	0	23.4	16
P. fuscus	1	20.3	31
P. chrysogenum	1	22.8	34
P. expansum	3	20.0	42
P. glabrum	7	19.1	49
P. viridicatum	13	18.9	49
P. terrestre	5	19.0	55
P. chloroleucon	22	21.6	56
P. viridicatum	6	21.9	56
P. terrestre	14	18.0	62
Penicillium[2]	16	21.0	62
P. expansum	13	14.0	69
P. viridicatum	24	18.3	70
Penicillium[2]	23	18.8	71
P. chloroleucon	21	18.0	72
P. cyclopium	22	17.9	73
P. Trzebinskii	23	17.6	73
P. expansum	23	16.4	75
Penicillium[2]	23	16.9	78
P. cyclopium	22	11.9	78
Penicillium[2]	19	13.7	81
P. viridicatum	29	12.7	87
P. rugulosum	37	11.1	95
P. chrysogenum	38	9.0	97
H. sativum alone	22	18.1	73
Wheat alone[3]	0	22.2	0
Isolates alone[4]	0	20.3	0

Isolate	Dead plants	Av. ht., cm.	Av. rt.,[1] %
Miscellaneous fungi			
Trichoderma viride	0	23.1	4
T. lignorum	0	23.0	6
T. lignorum	0	23.9	12
Absidia glauca	2	22.3	23
Aspergillus nidulans	10	18.7	32
Mucor hiemalis	19	20.6	57
Rhizopus sp.	18	22.2	55
Rhizopus sp.	18	16.6	62
Aspergillus niger	22	20.1	65
H. sativum alone	13	18.4	62
Wheat alone[3]	0	22.0	0
Fungi alone[4]	0	22.7	0
Actinomyces			
Actinomyces 2	0	22.3	33
Actinomyces 3	12	24.6	44
Actinomyces 6	12	24.9	44
Actinomyces 7	17	25.2	55
Actinomyces 5	23	28.0	65
Actinomyces 4	25	25.9	70
Actinomyces 1	29	24.5	78
Actinomyces 8	26	22.9	82
H. sativum alone	21	22.3	66
Wheat alone[3]	0	24.2	0
Isolates alone[4]	0	26.4	0

[1] *Disease rating.*
[2] *Species not identified.*
[3] *No micro-organisms present in soil.*
[4] *Average effect of isolates as tested separately, and the pathogens absent.*

four remaining unidentified isolates one depressed virulence by 11%, two had no definite effect, and one increased virulence slightly.

Miscellaneous Fungi

Of the miscellaneous fungi tested, the depressing effect of *Trichoderma* spp. on the virulence of *H. sativum* (Table I) was very great. An isolate of *T. viride* (Pers.) ex Fries, reduced the infection rating by 58%, and two of *T. lignorum* (Tode) Harz. reduced it by 56 and 50%, respectively. A random isolate of *Absidia glauca* Hagem, reduced infection 39%, and one of *Aspergillus nidulans* (Eidam) Wint. by 30%. The effect of *A. niger* v. Tieg., *Rhizopus* sp., and *Mucor hiemalis* Wehm., was not appreciably different from that of *H. sativum* alone.

Actinomyces spp.

In comparison with a disease rating of 66% for *H. sativum*, four of the eight isolates of *Actinomyces* depressed the virulence of the pathogen by 33, 22, 22, and 11%, respectively; two had no marked effect; and the other two appeared to increase it by 12 and 16%, respectively (Table I).

Discussion

The foregoing experiments have demonstrated that a fairly high percentage of random isolates of the genus *Penicillium* are quite antagonistic to the virulence of *H. sativum* on wheat seedlings, and also that certain random isolates within a given species may be very antagonistic, while others are not. Other work has shown that certain isolates of *Penicillium* reduced the virulence of *Pythium debaryanum* (5) and of *Ophiobolus graminis* (3, 4). Asthana (1) found that, of several fungi used, *Penicillium chrysogenum* was among those that exerted the greatest repressive effect on the growth and parasitic vigour of *Botrytis cinerea*. It is obvious from the present studies that within a given species some isolates may exert no noticeable effect on the virulence of the pathogen, others may depress it, and some actually increase its virulence. Possibly this ability to increase the virulence of a pathogen is even more common among micro-organisms than would appear from the data presented. An increase of virulence of *O. graminis* by the action of certain associated fungi was reported in 1931 by Sanford and Broadfoot (4), and Borzini (2) reported a similar case in 1937.

Obviously it is important to know the relative antagonistic effect on a given pathogen of the various genera and species of fungi that inhabit the soil, but it would appear from these studies that it is even more essential to ascertain the range of variation of this kind exhibited by random isolates of a given species. In other words, the behaviour of a single isolate may be quite different from the average for the species. The interesting question now is whether the plant pathologist, in controlled greenhouse or ordinary field experiments, is more concerned with the average association effects of a given species than with those of particular isolates or groups of them. At least it is reasonable to suppose that in rigidly controlled experiments such as are reported here there would be instances where the effects of one isolate counterbalance those of another, and that in natural soil the combined effects of other micro-organisms might produce a different result. It is regretted that more complete data on this general question cannot be supplied at this time.

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VEGETATIVE PROPAGATION OF CONIFERS

VII. OUTDOOR PROPAGATION OF A NOVEMBER COLLECTION OF NORWAY SPRUCE CUTTINGS TREATED WITH PHYTOHORMONES, CANE SUGAR, AND AN ORGANIC MERCURIAL DISINFECTANT[1]

By N. H. Grace[2], J. L. Farrar[3], and J. W. Hopkins[4]

Abstract

Dormant Norway spruce cuttings were collected in mid-November and treated with talc dusts containing two separate phytohormone chemicals, indolylacetic and naphthylacetic acids, in three concentrations, 0, 1000, and 5000 p.p.m., alone, and in combination with cane sugar and an organic mercurial disinfectant. Treated cuttings were planted immediately in outside frames in sand and a mixture of sand and peat in equal proportions. They were removed for examination 10 months later. Phytohormone treatment, except with the 5000 p.p.m. concentration of naphthylacetic acid, which was injurious, increased the number of cuttings rooted, the number and length of roots, the number of surviving cuttings, and the number of rooted cuttings with new growth. There were also marked effects on the initiation and development of new growth. The admixture of peat in the propagation medium improved rooting and development of new growth, particularly in certain of the phytohormone treatments. Only about 50% of the controls rooted in both sand and sand-peat, while the 5000 p.p.m. concentration of indolylacetic acid effected 68% rooting in sand and 82% in sand-peat.

Both cane sugar and organic mercury significantly affected a number of the responses. However, the effects were comparatively small and depended, for the most part, on interactions with phytohormone treatments and media. Organic mercury increased the number of cuttings rooted by about 6%.

Introduction

Although special consideration has been given to the rooting of cuttings of Norway spruce in the course of studies on the vegetative propagation of conifers by several workers (3, 6, 8, 11, 14, 18), much of the experimental work so far reported has dealt with propagation in the greenhouse. While this method of approach may contribute valuable physiological information, it also has to be recognized that methods of propagation in outside frames must be perfected before vegetative propagation can be applied in forestry on any large scale.

This communication accordingly describes an experiment in which dormant Norway spruce cuttings were taken in November and planted in outside frames. The experiment involved the use of a series of dust preparations containing plant hormones and other chemicals previously shown to have some influence on the rooting of cuttings (7, 9). The dust method of applying

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[2] *Biochemist, National Research Laboratories, Ottawa.*

[3] *Assistant in Forestry and Laboratory Research, Dominion Forest Service, Ottawa.*

[4] *Statistician, National Research Laboratories, Ottawa.*

the chemicals was chosen in preference to the solution method, which has failed, in the experience of the authors, to have beneficial effects on the rooting of cuttings of this species of spruce (3, 4, 10, 11). The experiment was made in two media, sand and a mixture of 50% sand and 50% peat humus by volume, and was designed to permit consideration of the effects of the various chemicals individually, in combination, and in relation to the two media employed.

As a previous paper has already dealt with similar Norway spruce cuttings collected at the same time and treated with the same dusts, but propagated during the winter in a greenhouse (9), it is also possible to draw some broad comparisons between outside and greenhouse methods of propagation of cuttings of this species.

Experimental

Branches were collected from the upper half of Norway spruce trees 18 years old in a plantation situated at the Petawawa Forest Experiment Station, Chalk River, Ontario. Cuttings without a heel of old wood, and ranging from 2 to 3.5 in. in length, were rapidly taken with secateurs from twigs less than 8 in. long. Each group of 30 cuttings, the unit in the experiment, was representative of the different lengths. There were approximately four lateral cuttings to one terminal. Preparation, treatment, and planting of the cuttings was completed on November 15, 1938, the day the branches were obtained.

The series of dust preparations (9) contained the two phytohormones indolylacetic and naphthylacetic acids, each separately, at three concentrations, namely, 0, 1000 and 5000 p.p.m. (p.p.m. of hormone in hormone talc mixture), in conjunction with cane sugar at 0 and 10% and ethylmercuric phosphate at 0 and 50 p.p.m., all in talc. There were thus 20 different preparations in this factorial series of dusts. The freshly prepared cuttings were dipped without dampening to a depth of about $\frac{3}{4}$ in. into the appropriate dust in groups of 30 and planted in four replicate randomized blocks. Each of the four replicate blocks also contained four duplicates of the treatments with talc, sugar, and organic mercury, in order to provide averages for cuttings with no phytohormone treatment of the same weight as those for each hormone dosage when averaged over both chemicals. The entire experiment required 2880 cuttings.

Two contiguous 3.5 by 24 ft. propagation frames shaded by screens of lath covered with factory cotton were used. One accommodated two complete replications planted in sand, and the other, two replications in the mixture of sand and peat humus.

The sand employed was fine and quite uniform, 91% passing through a 1 mm., and being held by a 0.05 mm., sieve. Its clay fraction was about 2% and organic matter content less than 1%. A volume of 100 cc. held 27 cc. of water against gravity. The reaction of the sand was slightly acid, with pH ranging from 6.2 to 6.5.

The peat humus was obtained from Alfred, Ontario, through a commercial supply house. The products of this peat bog have been described elsewhere (12, 17). Analysis of the shipment used in this experiment showed the ash content to be 10.5%. About one-quarter of the ash was lime but only half of it was classified as exchangeable. Qualitative tests showed that nitrates were present but not nitrite or ammonia. Nitrifying bacteria were shown to be present, and the pH was found to be 6.8*. Because of its fine texture the peat humus mixed readily with the sand. A volume of 100 cc. of the mixture held 35 cc. of water against gravity.

Cuttings were planted at a depth of about 1.5 in. and were left in the frames continuously until mid-September, 1939, when they were removed for counts and measurements. From the date of planting until early in June they received no attention. After that date the frames were watered daily, the sand being maintained at a usual moisture content of 4 to 7 cc. and the peat-sand at 22 to 30 cc. per 100 cc. of medium. As the pore space per 100 cc. of medium was 40 cc. for sand and 67 cc. for the mixture of sand and peat, this left an air content of about 35 and 41 cc. respectively in the two media.

The air temperature in the frames rarely fell below 50° F. or rose above 100° F. during June, July, and August. The maximum temperature reached in the frame was 14° above that outside. Evaporation ranged from 20 to 60 Livingston units per week (16). This was about 20% of the evaporation from an exposed atmometer in the same locality, and about half of that recorded in a greenhouse at New Haven (3, 4). The composition of the medium had no apparent effect on air temperature or evaporation.

While imported granulated peat moss has been used by various workers in the propagation of cuttings, the domestic sedge peat employed in this experiment has been used by comparatively few (1, 13, 15, 19). The addition of any peat to sand will increase the amount of moisture held, and it is quite possible that this is an important effect. Peat also affects the aeration, acidity, and base exchange capacity. Its effect may also be due to its chemical constituents or to the microflora it contains.

Results

Statistical Treatment of the Data

In Table I are given the results of the analyses of variance of the counts and measurements made on the cuttings. Counts of the number of cuttings with new growth, terminal new growth, and the number of new growth shoots per group of 30 cuttings were made in early August and the data analysed as such. Data for the number of cuttings surviving at that time were subjected to the inverse sine transformation prior to analyses of variance (2), as were all counts made in September, when the cuttings were taken up. Succeeding tables illustrate, not all the statistically significant differences, but a selection of the more important effects and interactions of the chemical treatment and propagation medium.

* *The analyses on the peat humus used in this experiment were carried out by Dr. C. C. Heimburger, Dominion Forest Service, Ottawa.*

TABLE I

ANALYSIS OF VARIANCE OF RESPONSES OF NORWAY SPRUCE CUTTINGS TREATED WITH TALC DUSTS CONTAINING PHYTOHORMONES, CANE SUGAR, AND ORGANIC MERCURY, AND PROPAGATED IN SAND AND SAND-PEAT

Source of variance	Degrees of freedom	Mean square										
		Number of cuttings rooted	Number of roots per cutting rooted	Length of root per rooted cutting	Mean root length	Number of surviving cuttings		Number of cuttings with		Number of new growth shoots per group of cuttings	Number of rooted cuttings with new growth	Per cent of rooted cuttings (without new growth —with new growth)
						August	September	New growth	Terminal new growth			
Replicates												
Between media	1	1123.5	0.41	417.9	31.21	92.6	2.5	442.0***	615.1***	1683.4***	4198.9*	12398.8*
Within media [Error *(a)*]	2	79.1	2.76	28.7	3.79	20.6	35.5	2.2	6.5	0.9	82.2	654.0
Sugar	1	191.0	0.03	5.0	0.20	297.2*	516.2*	88.2**	106.3**	216.0**	229.1**	19.3
Mercury	1	238.1*	7.21*	93.4**	0.88	75.1	26.5	3.4	0.3	45.4	132.3*	250.3
Sugar × mercury	1	39.8	2.13	0.8	0.97	77.9	15.7	28.2	14.3	26.0	145.3*	250.3
Phytohormone												
Dosage	2	507.8**	4.12*	12.1	0.35	1091.4***	2450.4**	382.6***	335.7***	503.6***	304.0***	6598.4***
Chemical	1	5672.0**	14.34**	59.1**	0.14	2907.9***	9560.0**	915.1***	708.9***	2743.2***	3284.7***	8649.0***
Chemical × dosage	1	5781.7**	40.6	85.3*	1.27	1260.3***	5073.0**	400.0***	319.5***	791.0***	2741.8***	1936.0
Phytohormone × sugar												
Dosage × sugar	2	6.7	1.18	2.7	0.07	479.1**	600.9**	10.4	6.6	44.0	32.2	659.1
Chemical × sugar	1	209.2	1.79	0.4	0.06	173.6	141.6	52.6*	40.6	107.6*	123.5	3.1
Dosage × chemical × sugar	1	614.4**	0.58	0.1	0.03	208.8	329.4*	0.3	0.1	34.5	147.9*	315.1
Phytohormone × mercury												
Dosage × mercury	2	143.4	0.62	1.7	0.25	317.1**	192.0	2.4	1.5	27.8	14.7	260.0
Chemical × mercury	1	38.9	1.13	26.7	0.74	15.1	355.3*	0.6	5.6	26.3	29.6	529.0
Dosage × chemical × mercury	1	53.5	1.65	0.9	0.06	26.0	0.5	2.3	5.6	43.9	0.9	4.0
Phytohormone × sugar × mercury												
Dosage × sugar × mercury	2	145.9	4.11*	5.8	0.30	26.5	37.9	9.3	13.0	14.5	186.3**	2351.1*
Chemical × sugar × mercury	1	0.4	3.56	0.6	0.46	76.1	141.0	4.0	3.5	23.8	0.4	1388.8
Dosage × chemical × sugar × mercury	1	16.3	2.15	24.9	2.81**	39.7	44.6	15.6	0.8	54.4	12.9	661.8
Sugar × media	1	175.5	1.58	2.2	0.13	19.7	105.8	6.0	11.4	22.0	404.7**	810.8
Mercury × media	1	72.8	0.21	50.6	1.34	37.6	160.7	1.0	10.0	16.7	38.4	86.3
Sugar × mercury × media	1	7.6	1.15	4.3	1.34	152.3	235.2	8.2	11.3	0.7	4.6	162.8

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*

TABLE I—*Concluded*

ANALYSIS OF VARIANCE OF RESPONSES OF NORWAY SPRUCE CUTTINGS TREATED WITH TALC DUSTS CONTAINING PHYTOHORMONES, CANE SUGAR, AND ORGANIC MERCURY, AND PROPAGATED IN SAND AND SAND-PEAT—*Concluded*

Source of variance	Degrees of freedom	Mean square										
		Number of cuttings rooted	Number of roots per cutting rooted	Length of root per rooted cutting	Mean root length	Number of surviving cuttings		Number of cuttings with		Number of new growth shoots per group of cuttings	Number of rooted cuttings with new growth	Per cent of rooted cuttings (without new growth —with new growth)
						August	September	New growth	Terminal new growth			
Phytohormone × media												
Dosage × media	2	624.1**	9.21**	67.1**	0.08	242.8*	408.4**	3.6	7.5	2.8	69.8	2312.3*
Chemical × media	1	0.1	11.46**	0.8	1.54*	258.5*	367.7*	100.0**	102.5**	23.8	79.9	4.0
Chemical × dosage × media	1	76.4	10.33**	52.0	0.06	119.9	42.6	3.1	17.0	62.0	13.4	225.0
Phytohormone × sugar × media												
Dosage × sugar × media	2	68.5	4.79*	18.3	0.07	48.6	23.4	4.9	4.0	5.0	24.9	372.8
Chemical × sugar × media	1	4.4	9.66**	61.4*	0.27	8.1	0.8	1.0	1.9	0.8	40.5	1139.1
Dosage × chemical × sugar × media	1	45.4	2.03	1.0	1.19	62.8	0.0	1.6	5.6	15.0	21.5	162.6
Phytohormone × mercury × media												
Dosage × mercury × media	2	78.6	1.96	28.1	0.03	61.3	2.9	1.0	1.1	4.2	78.1*	542.5
Chemical × mercury × media	1	165.4	0.00	1.7	0.12	2.7	359.1*	6.3	1.9	0.1	38.9	100.0
Dosage × chemical × mercury × media	1	28.2	0.40	0.4	0.00	86.0	13.7	10.6	15.0	1.9	9.5	256.0
Phytohormone × sugar × mercury × media												
Dosage × sugar × mercury × media	2	129.7	1.14	18.7	0.16	98.2	214.2	8.5	7.0	45.5	101.9*	345.2
Chemical × sugar × mercury × media	1	147.3	0.24	0.3	0.15	51.5	189.8	2.3	0.4	1.9	116.9*	297.6
Dosage × chemical × sugar × mercury × media	1	62.6	1.13	65.4*	0.87	71.0	72.6	5.1	8.3	19.2	40.5	39.1
Error *(b)*	54	56.1	1.12	13.0	0.37	61.1	72.7	10.3	10.9	24.0	24.6	545.6

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*

Number of Cuttings Rooted

Table II shows the average effects of phytohormone dust treatments and media on the number of cuttings rooted. The results given are averages for treatment with and without cane sugar and organic mercury. In sand, the 5000 p.p.m. concentration of indolylacetic acid increased rooting whereas this concentration of naphthylacetic acid markedly reduced it. The 1000 p.p.m. concentration of both hormones was without effect. In the mixture of sand and peat, rooting of the controls was of the same order as in sand only. However, each treatment resulted in substantially better rooting in the mixture than did the same treatment in sand. Both concentrations of indolylacetic acid and the 1000 p.p.m. concentration of naphthylacetic acid increased the number of cuttings rooted. The 5000 p.p.m. concentration of naphthylacetic acid reduced rooting, but not to the same extent as in sand. The 5000 p.p.m. level of indolylacetic acid—the best treatment—effected 82% rooting in sand-peat, while the controls without hormone rooted about 48%.

TABLE II

AVERAGE EFFECTS OF PHYTOHORMONE DUST TREATMENTS AND MEDIA ON THE NUMBER OF CUTTINGS ROOTED

Medium	Data	Phytohormone concentration in talc, p.p.m.				
		0	Indolylacetic acid		Naphthylacetic acid	
			1000	5000	1000	5000
Sand	Transformed	46.8	42.8	56.1	44.8	15.6
	Per cent	52.9	46.3	68.3	49.6	11.7
Sand-peat	Transformed	43.4	56.5	65.4	54.9	30.2
	Per cent	47.5	68.1	82.1	66.3	26.7

Necessary difference, 5% level, between controls and treatments 6.5, treatments 7.5 (transformed data).

Interaction effects of phytohormone and cane sugar dust treatments are shown in Table III. The data are averages for treatment with and without organic mercury and for both media. The combination of sugar with indolylacetic acid caused a reduction in the number of rooted cuttings at the 1000, but a slight increase at the 5000, p.p.m. concentration. Rooting tended to be reduced at 1000 p.p.m. naphthylacetic acid in the presence of sugar and this reduction reached significance at the 5000 p.p.m. level. It is apparent that the beneficial effects of 1000 p.p.m. naphthylacetic acid, given in Table II, were obtained in the absence of sugar.

Rooting was increased by the treatment with 50 p.p.m. of organic mercury from 48.8 to 54.5%—the figures being averages over all other treatments.

Number of Roots per Rooted Cutting

The average of the number of roots per rooted control cutting was 4.1. Phytohormone treatment increased this number by approximately one.

Indolylacetic acid was somewhat more effective than naphthylacetic acid, and the stimulatory effect of hormone treatment was more marked in sand-peat than in sand.

TABLE III

AVERAGE EFFECTS OF PHYTOHORMONE AND CANE SUGAR DUSTS ON THE NUMBER OF CUTTINGS ROOTED

Concentration of sugar, %	Data	Phytohormone concentration in talc, p.p.m.				
		0	Indolylacetic acid		Naphthylacetic acid	
			1000	5000	1000	5000
0	Transformed	43.9	53.2	59.0	50.8	31.0
	Per cent	48.1	62.9	72.5	59.2	27.9
10	Transformed	46.3	46.1	62.5	48.9	14.8
	Per cent	52.3	51.7	77.9	56.7	10.4

Necessary difference, 5% level, between controls 5.3, controls and treatments 6.5, treatments 7.5 (transformed data).

Total Root Length per Rooted Cutting

The average total root length per rooted cutting was about 8.5 cm. in sand and 13.0 cm. in sand-peat. No significant increase in total root length due to treatment was demonstrable in sand, but in sand-peat, the 15.7 cm. length observed on the rooted cuttings treated with 5000 p.p.m. indolylacetic acid was significantly greater than the value of 11 cm. for the controls. On the average, organic mercury treatment increased the length of root from 9.5 to 11.5 cm.

Mean Root Length

The mean root length was about 2.5 cm. in both media. Of the various chemical treatments, the 1000 p.p.m. concentration of naphthylacetic acid resulted in the greatest mean root length, 3.0 cm. in the presence of organic mercury and the absence of cane sugar, when averaged over both media. The statistical analysis also revealed, however, that whereas naphthylacetic acid treatment resulted in a greater mean root length in sand than indolylacetic acid treatment, in sand-peat mean root length was greater after application of indolylacetic acid.

Number of Surviving Cuttings

Observations in early August indicated an average survival of 92.5%. Despite this high average, the data demonstrated increased survival from indolylacetic acid treatments and the 1000 p.p.m. concentration of naphthylacetic acid. While sugar treatment increased survival of the controls without phytohormone, in combination with the 5000 p.p.m. concentration of naphthylacetic acid it was injurious. Similarly, organic mercury was beneficial in the absence of phytohormone but injurious with the highest concentration of naphthylacetic acid. For the most part, there was no significant difference

between media, but the injurious effects of 5000 p.p.m. naphthylacetic acid were reduced in sand-peat.

Average survival was reduced to 77.6% at the September count. The effects of both sugar and organic mercury treatments were essentially the same as in the earlier observations. The 5000 p.p.m. concentration of naphthylacetic acid resulted in survival of 50% in the absence, and 18% in the presence, of cane sugar. Organic mercury, similarly, accentuated the injurious effects of the highest concentration of naphthylacetic acid. However, in the absence of phytohormone, organic mercury increased survival from 76 to 84%. Survival after treatment with mercury in combination with both concentrations of indolylacetic acid and the 1000 p.p.m. concentration of naphthylacetic acid was not significantly greater than after organic mercury or phytohormone alone.

TABLE IV

AVERAGE EFFECTS OF PHYTOHORMONE DUST TREATMENTS AND MEDIA ON THE NUMBER OF CUTTINGS SURVIVING IN SEPTEMBER

Medium	Data	Phytohormone concentration in talc, p.p.m.				
		0	Indolylacetic acid		Naphthylacetic acid	
			1000	5000	1000	5000
Sand	Transformed	68.7	75.4	78.2	65.6	29.5
	Per cent	84.8	92.5	93.8	82.1	27.1
Sand-peat	Transformed	60.9	77.5	75.3	74.0	39.5
	Per cent	75.0	92.9	92.1	89.6	41.2

Necessary difference, 5% level, between controls and treatments 7.4, treatments 8.5 (transformed data).

Table IV indicates the effects of media and phytohormone dust treatments on the number of surviving cuttings in September. Data are averages for treatments with and without cane sugar and organic mercury. Whilst the 5000 p.p.m. concentration of indolylacetic acid was the only treatment to increase survival in sand, and all but the 5000 p.p.m. concentration of naphthylacetic acid increased survival in sand-peat, it requires to be noted that the survival of the controls was lower in the second medium. The injurious effect of the high naphthylacetic dosage was however less evident in sand-peat. Although indolylacetic acid treatments in both media resulted in survival of the order of 93%, comparison with the data of Table II indicates that a substantially greater percentage of the surviving cuttings rooted in sand-peat than in sand only.

Development of New Growth

New growth, which developed on only 54% of the cuttings, began after the general appearance of growth on the parent tree and continued throughout most of the summer.

Effects of treatments with phytohormones and cane sugar on the number of cuttings with terminal new growth are given in Table V, in which the data are averages for treatments with and without organic mercury and in both media. The average effect of sugar was a decrease in the number of cuttings with terminal new growth, and that of phytohormone treatment an increase with 1000 p.p.m. indolylacetic acid. and a decrease following the highest concentration of naphthylacetic acid.

TABLE V

AVERAGE EFFECTS OF PHYTOHORMONE AND CANE SUGAR DUST TREATMENTS ON THE NUMBER OF CUTTINGS WITH TERMINAL NEW GROWTH

Cane sugar concentration, %	Data	Phytohormone concentration in talc, p.p.m.					Means for sugar treatments
		0	Indolylacetic acid		Naphthylacetic acid		
			1000	5000	1000	5000	
0	Average of 30 cuttings	17.7	18.2	17.5	17.6	8.0	16.1
	Per cent	59.0	60.8	58.3	58.8	26.7	53.8
10	Average of 30 cuttings	15.2	18.8	16.4	15.0	3.6	14.0
	Per cent	50.6	62.5	54.6	50.0	12.1	46.7
Means for treatments	Average of 30 cuttings	16.4	18.5	16.9	16.3	5.8	
	Per cent	54.8	61.7	56.5	54.4	19.4	

Necessary difference, 5% level, between controls without phytohormone 2.4, controls and treatments 2.9, treatments 3.3, controls and treatment means 2.0, treatment means 2.4 (averages of 30).

The effect of media, averaged over all other treatments, was highly significant, 41.8% of the cuttings in sand against 58.7% of those in sand-peat developing terminal new growth.

Whereas 50% of the cuttings showed terminal growth, an additional 4% had lateral new growth without terminal. Inclusion of these latter had little effect on the treatment differences shown in Table V, but did result in the demonstration of a significant reduction in the total number of cuttings with new growth following treatment with both concentrations of naphthylacetic acid in combination with cane sugar.

Data for the effects of phytohormone and cane sugar dust treatments on the average number of new growth shoots per group of 30 cuttings are given in Table VI. These are averages for treatments with and without organic mercury and in both media. It is apparent that treatment effects were similar to those indicated in Table V. There was also a significant difference between media in this respect. In the mixture of peat and sand there were on the average 25.5 shoots per group, as compared to only 17.1 in sand. The beneficial effects of the mixture of sand-peat were also seen in the length of new growth produced. Random selection of 121 cuttings propagated in sand gave an average length of 2.2 cm. per shoot, while the average for 146

cuttings from sand-peat was 4.0 cm.. Thus, the addition of peat increased the length of growth as well as the number of shoots and the number of cuttings producing growth.

TABLE VI

AVERAGE EFFECTS OF PHYTOHORMONE AND CANE SUGAR DUST TREATMENTS ON THE NUMBER OF NEW GROWTH SHOOTS PER GROUP OF 30 CUTTINGS

Cane sugar concentration, %	Phytohormone concentration in talc, p.p.m.				
	0	Indolylacetic acid		Naphthylacetic acid	
		1000	5000	1000	5000
0	23.8	26.3	29.0	24.3	10.0
10	19.7	30.0	25.5	19.9	4.3

Necessary difference, 5% level, between controls without phytohormone 3.3, controls and treatments 4.0, treatments 4.6.

Number of Rooted Cuttings with New Growth

The effects of phytohormone and cane sugar treatments on the number of rooted cuttings with new growth were similar to those given in Table III. Of the total number of rooted cuttings, the proportion with new growth ranged from about 50% for the controls to 60% for the indolylacetic acid treatments. The treatment with 1000 p.p.m. naphthylacetic acid in the absence of sugar resulted in 61% of the rooted cuttings having new growth.

On the average, there was an increase in the number of rooted cuttings with new growth following treatment with organic mercury in the absence, but not in the presence, of cane sugar, but at 5000 p.p.m. of both hormones organic mercury was beneficial in the absence, but detrimental in the presence, of sugar. In conjunction with the 1000 p.p.m. concentration of both hormones, organic mercury treatment increased the number of rooted cuttings with new growth by about 5%, and the addition of sugar had no effect. The beneficial effect of organic mercury in the case of controls without phytohormone was greater in sand-peat than in sand in the absence of sugar. This difference between media disappeared in the presence of sugar. In conjunction with 5000 p.p.m. naphthylacetic acid, on the contrary, the effect of organic mercury was greater in sand than in sand-peat. This difference also vanished on the addition of sugar.

While only 19.4% of the total of cuttings planted were rooted and had new growth in sand, the corresponding percentage was 38.0 in sand-peat.

Incidence of New Growth in Relation to Subsequent Rooting

Study of the relation of media and phytohormone treatments to the effect of new growth on rooting revealed that the main hormone effect in sand was due to the 5000 p.p.m. concentration of naphthylacetic acid. After this treatment the presence of new growth favoured rooting, whereas both the

controls and other treatments were followed by better rooting of the cuttings without new growth. In sand-peat the 1000 p.p.m. concentration of indolylacetic acid favoured rooting of cuttings without new growth. This was the only significant effect. On the average, new growth was harmful to rooting in sand and had no effect in sand-peat. Over the entire experiment 12.3% more of the cuttings without new growth were rooted than of those with such growth.

Discussion

Outside propagation of this November collection of Norway spruce cuttings resulted in much better rooting than was obtained in the greenhouse with cuttings of the same collection, although it is true that the propagation media were not identical (9). While the earlier experiment indicated a beneficial effect from 1000 p.p.m. indolylacetic acid, which effected rooting of 42%, an increase of about 10% above the controls, the 5000 p.p.m. concentration and both concentrations of naphthylacetic acid either had no effect or were injurious. In the present experiment, however, all the phytohormone treatments but the 5000 p.p.m. naphthylacetic acid had a marked beneficial effect. It is clear therefore that the results of greenhouse experiments cannot in all cases be used readily to evaluate the effectiveness of phytohormone treatments under outside conditions.

The beneficial effects of the sand-peat medium were an outstanding feature of the results. In the main, these related to the interactions between phytohormone treatments and the medium. However, differences between media were marked in relation to effects on new growth. The substantial stimulation of new growth in sand-peat may be related to nutrient factors from the peat, since nutrient salts applied to a sand medium have been shown to affect new growth by the cuttings (6). It is particularly interesting to note that the combination of sand-peat with certain phytohormone treatments resulted in marked increase in the number of cuttings rooted and, at the same time, an increase in both the number and length of new growth shoots. High percentage rooting and vigourous top growth are of obvious importance in the development of spruce plantations by means of vegetative propagation.

The beneficial effects resulting from treatments with cane sugar and, more particularly, organic mercury are similar to those noted in earlier experiments (7, 9). However, for the most part, these effects were comparatively small and depended on interactions with phytohormone treatments, particularly the high concentration of naphthylacetic acid.

Greenhouse experiments have indicated that the best rooting is obtained with early winter collections of dormant material. The present November collection likewise gave much better rooting than was obtained on the outside propagation of an April collection of dormant cuttings (10).

It has been stated that the cuttings should preferably be moistened prior to dusting if appreciable effects are to be obtained from phytohormone treat-

ment (14). This conclusion appears rather too general since the cuttings in this experiment were not moistened prior to dry dusting.

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YIELDING CAPACITY OF WHEAT CROSSES AS INDICATED BY BULK HYBRID TESTS[1]

By J. B. Harrington[2]

Abstract

In order to determine readily the usefulness of heterosis as an indicator of the yielding capacity of cereal crosses, replicated half rod row yield trials were made with bulk unselected seed of ten wheat crosses in F_2 and of six in F_3. The practical yielding value of the latter six crosses was ascertained later by replicated rod row yield tests of selected lines in F_6, F_7 and F_8. All the F_2 populations (excepting a *T. vulgare* × *T. persicum* cross) yielded significantly more than the mean of the parental varieties, the increased yield being 52 and 64% for two of the crosses. Of the six F_3 populations tested, the only one to yield significantly above the parental average was Reward × Hope, and the only ones to yield significantly less than the parental average were the two having Quality as one parent. Replicated plot tests of purified lines selected for high yield and other agronomic virtues showed the Reward-Hope combination to be particularly valuable, whereas the two crosses containing Quality were unpromising. The results suggest that replicated bulk F_2 tests may be used to indicate the yielding potentialities of wheat crosses, and that bulk F_3 tests may have supplementary value in this regard.

The breeder of cereal crops makes many crosses, each with a definite purpose, but he has no sure way of knowing their comparative values in advance. This is particularly true respecting the very important attribute "yielding capacity".

It has been established that certain varieties have a higher value in combining ability than others. This is illustrated by recent work in barley and oats. Harlan, Martini, and Stevens (6) carried 379 barley crosses separately in unselected mass for seven years and recorded the yields of single 10-ft. rows. They found that some crosses yielded much more than others and that the average yield of selections from the higher yielding crosses decidedly surpassed the yields of selections from the lower yielding crosses. Coffman *et al.* (2) showed the oat variety Markton to be a better parent than several other varieties.

An outstanding advance in the development of tests designed to reveal superior combining ability is the development of the "top crossing" principle. Lindstrom (10) announced this principle in 1931 as a valuable technique in maize improvement. Jenkins (9) found that crosses of inbred lines of corn with a commercial variety (top crossing) could be used satisfactorily for testing the yielding value of the selfed lines as parents in single and double crosses.

Studies on the intricate yield mechanism of plants have been made, by Bridgeford and Hayes (1), Waldron (13), and many others, but with all this work the breeder was not furnished with a safe rapid method of predicting the probability of obtaining high yielding segregates from a given cross.

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Contribution from the Field Husbandry Department, University of Saskatchewan, Saskatoon, Sask.

2 *Professor of Field Husbandry.*

An approach to the subject was made by the writer (7) in 1932 in a paper on predicting the value of a cross from an F_2 analysis. In that paper the writer concluded that the data on several hundred individual F_2 plants gave a reasonably accurate prediction of the yielding value of the wheat cross Marquillo × Marquis which was studied. This attack on the problem was satisfactory but it had two disadvantages, one being the cost of detailed analyses of hundreds of F_2 plants and the other the lack of direct comparison with other crosses.

Coffman and Wiebe (3), in an unreplicated rod row test in 1938, found that F_2 populations outyielded the average of the parent varieties in three out of four oat crosses studied. No attempt was made to interpret the results in terms of the relative yielding capacities of the crosses.

Since yield depends upon many genetic factors, it is reasonable to assume that the probability of obtaining high yielding segregates would be greater in a cross between parents with a larger total of yield genes than in one between parents possessing relatively few such genes. If this assumption is correct, heterosis, as shown by the extent to which the earliest hybrid generations excel the parental varieties in yield, should be a true indication of the yield value of a cross. In 1930 the writer began a study in which F_2 and F_3 bulk populations were given replicated plot tests to furnish information on the relation between heterosis and the yielding value of different varietal combinations in wheat. The F_1 was not used owing both to the cost of producing sufficient hybrid seed for adequate plot tests and to the difficulty of making satisfactory comparative yield tests of individual plants of the F_1 and the parental varieties.

Materials and Methods

Six crosses of compatible varieties differing in yielding ability were made in 1930 and the unselected bulk F_2 and F_3 generations of each cross were placed in replicated half-rod-row yield trials in 1932 and 1933 along with the parental varieties. The crosses* were as follows: Quality × Ceres, Reward × Ceres, Quality × Reward, Reward × Hope, Garnet × Reward and Reward × Reliance.

In order to confirm or disprove the conclusions drawn from the F_2 and F_3 tests with regard to the comparative yielding capacities of the different crosses, standard six replicate rod row trials were made on selections from the crosses as soon as reasonably true breeding lines were obtained. These

* *Following are the pedigrees of the varieties used in the crosses (S means Saskatchewan Accession Number, C means Canadian Accession Number): Apex (S1789, C1909) from H-44-24 (sib of Hope) × Double Cross (sib of Thatcher) × Marquis; Black Persian (S1801, C1238) introduction typical of T. persicum; Bobs (Sask. 1688, C1665) supposedly from a cross of Nepal barley with Early Lambrigg wheat; Ceres (S1212, C1263) Kota × Marquis; Garnet (S791, C1316) Riga (Ladoga × Red Fife) × Preston (Onega × Gehun); Hope (S1663, C1339) Vernal emmer × Marquis; Marquis (S100, C1691) Red Fife × Hard Red Calcutta; Mayview (S1286, C1694) a compactum selection from Fortyfold; Pioneer (S1472, C1467) sib of Garnet (Riga × Preston); Quality (S1200, C1490) probably sib of Florence (White Naples-Improved Fife × White Naples × Improved Fife-Eden); Reliance (S1851, C1498) Kanred (selection from Crimean) × Marquis; Reward (S1003, C1509) Marquis × Prelude (from Ladoga-White Fife × Hard Red Calcutta × Gehun); Thatcher (S1720, C1820) Kanred-Marquis × Iumillo durum × Marquis.*

tests were made on the lines from five of the crosses during the period 1936 to 1938. The sixth cross, Reward × Hope, was not continued at Saskatoon, largely on account of the extensive work with this combination of parents at another experiment station.

To furnish further information on the behaviour of different crosses a six replicate rod row plot test was made in 1939 of bulk F_2 of four crosses* ranging from a cross between closely related parents, Reliance × Thatcher, to a cross between Marquis and Black Persian *(T. persicum)* involving species of different chromosome number. The other crosses were Bobs × Pioneer, where the relationship is geographically wide, and Mayview *(T. compactum)* × Apex, a cross between related species of similar chromosome number.

The 1932, 1933 and 1939, tests were made according to the modified latin square of groups plan, each F_2 and its parental varieties constituting a group. The essentials of the method were described recently by Wishart (14). The plots were single rows eight feet long and one and a half feet apart. The number of replicates was four in 1932, three in 1933 and six in 1939.

The 1936, 1937 and 1938 tests were made according to the modified latin square plan. Triple rod row plots with rows one foot apart were used.

While a full record of plant height, days to heading, days to maturity, grain size, grain plumpness and several other agronomic attributes was obtained, it was considered that this paper could well be limited to a consideration of yield.

Results

The results from the six original crosses are presented in Table I. The F_2 of each of the crosses yielded significantly above the average of the parents. In four crosses the F_2 yielded significantly better than the higher yielding parent.

The F_3 yielded quite differently than the F_2. Only Reward × Hope F_3 significantly outyielded the parental variety average. The F_3 of both crosses involving Quality yielded significantly less than the parental variety averages.

The yield tests of selected purified lines in F_6, F_7 and F_8 showed no significant difference between parental variety average and line average in any cross. However, lines yielding as well as the higher yielding parent were obtained from Reward × Ceres, Garnet × Reward and Reward × Reliance. The Reward × Hope cross was discarded after the F_6 test, but lines significantly excelling both parental varieties in yield were obtained from this cross at the Dominion Experimental Farm, Brandon, Canada (11). Lines superior in yielding capacity were not obtained from the Quality × Ceres or Quality × Reward cross.

The results from the 1939 bulk F_2 yield test are given in Table II. The four crosses are arranged in order of estimated closeness of genetic relationship. The Thatcher × Reliance F_2 was significantly excelled by Reliance. The F_2 of Bobs × Pioneer, on the other hand, greatly excelled both parents. The same is true of the F_2 of Mayview × Apex only the contrast is more startling,

the F_2 yielding more than twice as much as Mayview. The Marquis × Black Persian cross gave a very poor F_2 yield owing to the high sterility of most of the segregates.

TABLE I

YIELDS FROM REPLICATED HALF ROD ROW TESTS OF WHEAT CROSSES IN UNSELECTED BULKED F_2 AND F_3 AND IN SELECTED LINES CARRIED IN REPLICATED ROD ROW TESTS IN F_6, F_7 AND F_8

Mean plot yld.	Quality × Ceres‡		Reward × Ceres‡		Quality‡ × Reward		Reward‡ × Hope		Garnet‡ × Reward		Reward × Reliance‡	
	Gm.	%	Gm.	%	Gm.	%	Gm.	%	Gm.	%	Gm.	%
F_2 bulk 1932	187	109	199	117	173	119	201	152	200	125	203	121
Low parent	147	85	143	84	143	99	121	92	143	90	143	85
High parent	197	116	197	116	147	101	143	108	176	110	193	115
Avg. parent	172	100	170	100	145	100	132	100	160	100	168	100
F_2-Avg. par.	15	9	29	17	28	19	69	52	40	25	35	21
Nec. diff.*	10		10		9		9		10		10	
F_3 bulk 1933	50	78	69	104	49	75	74	112	68	100	74	104
Low parent	63	98	67	102	67	103	64	97	67	98	67	94
High parent	64	100	64	97	63	97	67	102	68	100	75	106
Avg. parent	64	100	66	100	65	100	66	100	68	100	71	100
F_3-Avg. par.	−14	22	3	5	−16	25	8	12	0	0	3	4
Nec. diff.*	4		5		4		5		5		5	
Mean acre yld.	Bu.	%	Bu.	%	Bu.	%	Bu.	%	Bu.	%	Bu.	%
F_6 lines §1936			19.6	95					13.9	109	15.2	107
Low parent			22.6	110					12.5	98	14.5	102
High parent			18.5	90					13.0	102	13.8	97
Avg. parent			20.6	100					12.8	100	14.2	100
F_6-Avg. par.			−1.0	5					1.1	9	1.0	7
Nec. diff.*			3.9						2.0		1.4	
F_7 lines 1937	4.3	96	5.5	95	4.2	76			8.6x	92	3.2†	84
Low parent	4.9	109	6.7	116	5.8	106			9.7x	104	4.7	124
High parent	4.0	90	4.9	84	5.2	94			9.0x	97	3.0†	79
Avg. parent	4.5	100	5.8	100	5.5	100			9.3	100	3.8	100
F_7-Avg. par.	−0.2	4	−0.3	5	−1.3	24			−0.7	8	−0.6	16
Nec. diff.*	1.2		1.8		1.5				1.3		0.9	
F_8 best 2 lines			22.5	121					24.5	128	20.4	117
F_8 lines 1938			20.7	111					22.2	116	18.7	108
Low parent			17.4	94					17.4	91	17.4	100
High parent			19.8	106					21.0	109	17.3	99
Avg. parent			18.6	100					19.2	100	17.4	100
F_8-Avg. par.			2.1	11					3.2	17	1.3	8
Nec. diff.*			4.4						4.6		3.1	

NOTE: *The F_6 of Quality × Ceres and Quality × Reward was not tested until 1937. ‡The higher yielding parental variety in 1932. *Between hybrids and average of parents. †Later maturity greatly depressed the yields. xExtreme earliness considerably increased the yields. §The yields for lines are each averages of all lines tested. The numbers of lines averaged are as follows: Quality × Ceres 22 in F_6; Reward × Ceres 22 in F_6, 15 in F_7, 11 in F_8; Quality × Reward 8 in F_6; Reward × Hope 9 in F_6; Garnet × Reward 18 in F_6, 9 in F_7, 5 in F_8; Reward × Rel. 45 in F_6, 36 in F_7, 19 in F_8.*

TABLE II

REPLICATED ROD ROW TEST OF BULK F_2 AND PARENTAL VARIETIES OF FOUR CROSSES IN 1939

Variety or Hybrid	Sask. Acc. No.	Grain Yield in Bushels per Acre
Thatcher × Reliance F_2		43.9 ± 3.0
Reliance	1851	53.2 ± 3.7
Thatcher	1720	44.0 ± 3.0
Bobs × Pioneer F_2		49.8 ± 3.4
Bobs	1275	40.9 ± 2.8
Pioneer	1472	36.5 ± 2.5
Mayview × Apex F_2		54.9 ± 3.8
Apex	1789	41.7 ± 2.9
Mayview	1286	25.4 ± 1.8
Marquis × Black Persian F_2		11.2 ± 0.8
Marquis	100	47.3 ± 3.3
Black Persian	1801	38.0 ± 2.6

Source of variation	Deg. of freedom	Sum of squares	Mean squares	F value	5% point	1% point
Total	71	3060399	43104			
Between replicates	5	70182	14036	1.10	2.90	4.56
Between groups	3	496508	165503	13.00	3.29	5.42
Error *(a)*	15	190912	12728			
Between varieties	8	1853341	231668	20.62	2.18	2.99
Remainder, Error *(b)*	40	449456	11236			

Mean = 40.6 bu.
Within groups SE_v = 2.8 bu. Nec. diff. = 7.0 bu. SE_v = 6.9%.
Between groups SE_v = 2.8 bu. Nec. diff. = 7.1 bu. SE_v = 7.0%.

Discussion

It might be argued that heterosis as an indicator of yielding capacity would be unreliable since the dominance of genes for low yield might result in little or no heterosis, and crosses carrying such genes would be discarded as unpromising although they might have valuable recessive genes for high yielding capacity. While such a situation is possible, the writer finds no evidence to support it. Recently Hutchison (8) stated, in a paper on cotton crosses, that the dominance bias is in all cases in the direction in which recent selection has operated. If we accept this statement as having a wide application it would follow that most of the genes associated with high yielding capacity tend to be dominant, and that therefore the amount of heterosis as displayed by a hybrid population should be indicative of the total inherent yielding capacity of the hybrids. However, East (4), in presenting the theory of linked growth factors as being the most reasonable explanation of hybrid vigour, states that "universal experience is to find 'dominance', in the classical sense,

virtually absent". Apparently as far as the plant breeder is concerned the "dominance" concept does not need to be considered.

The complexity of heterosis, with its dependence on linkage, and the value of many characters other than those associated with growth make it difficult to provide from parental performance or in F_1, F_2 or F_3 a suitable method of estimating the probable value of a cross. Heterosis in a naturally self fertilized crop such as wheat is usually striking in F_1 and moderate in F_2. But some varietal combinations show no heterosis, and in others hybrid vigour may appear for some characters and not for others, as found by Rosenquist (12) in his study of hybrid vigour in wheat. The vigour importance of such characters as stooling propensity, height of plant, head length, seed size, and length of growth period varies with the material used. But, as East (4) points out, hybrid vigour is a phenomenon affecting the plant as a whole. And, as yield is a result of the operation of the plant as a whole, most investigators agree that increase in yield is a reliable index of the extent of heterosis.

The results in this paper, as well as in many other studies, indicate the dependence of heterosis upon the genetic differences of the parental stocks. It seems reasonable to expect a larger proportion of high yielding segregates from crosses involving parents with a high degree of genetic disparity than from crosses with more closely related parents. Therefore, it seems fair to assume that the amount of heterosis indicates the total inherent yielding capacity of a cross.

Considering the F_2 and F_3 results presented in Table I from this point of view, one would expect the Reward × Hope cross to be the most promising and the two crosses involving Quality to be the least promising. Actual results fully justify this prediction. The Hope-Reward combination produced many high yielding lines, whereas Quality × Ceres and Quality × Reward were so unpromising they were discarded after the F_6 tests.

If the results in Table II are interpreted in the same manner, it must be concluded, as far as yield is concerned, that there is no particular value in carrying on the cross Reliance × Thatcher. It must also be concluded that the crosses Bobs × Pioneer and Mayview × Apex have excellent yield possibilities.

While the results in this paper are from relatively few crosses they suggest that bulk F_2 yield trials may be used to indicate the potential yield possiblities of a cross. In addition the results in Table I suggest that F_3 yield trials are of supplementary value as a support to the F_2 conclusions.

Nevertheless, regardless of the usefulness of bulk hybrid tests in evaluating the yield potentialities of crosses, a number of economic characters other than yield may be limiting factors. Milling and baking quality, disease resistance, and resistance to certain weather conditions are some of these characters. For such characters the bulk hybrid trials may be of little use. For example, the cross Reliance × Thatcher (see Table II) involves two varieties of very high yielding capacity. The purpose of the cross is to combine the ability

of Reliance to produce a large, plump, heavy kernel with Thatcher's ability to resist stem rust. A bulk, F_2 test of this cross might well be considered of questionable value.

While the present study has furnished information of value on the usefulness of bulk hybrid trials in assaying the values of crosses, the writer feels that there is need for more extensive studies along the same line, not only in wheat but in other crops and that larger numbers of crosses should be involved in such studies. It would also appear to be worthwhile to have parental varieties F_1, F_2, and F_3 in the same replicated yield trial as suggested in 1932 by Fisher, Immer, and Tedin (5). Experiments along these lines are now under way on barley and wheat at a number of places, including Saskatoon.

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STUDIES ON THE BIONOMICS AND CONTROL OF THE BURSATE NEMATODES OF HORSES AND SHEEP

VIII. COMPARISONS OF THE LETHAL EFFECTS OF SOME CHEMICALS CONTAINING SULPHUR ON THE FREE-LIVING STAGES OF SCLEROSTOMES[1]

BY I. W. PARNELL[2]

Abstract

The effect on the free-living stages of sclerostomes of 14 common chemicals containing sulphur is discussed. Potassium xanthogenate in weak solution will sterilize about 160 times its weight of fresh faeces; in a strong solution, less than a quarter of the weight. Carbon disulphide will sterilize only about 32 times its weight under the conditions imposed by this technique. In solutions of medium strength the sulphates of zinc will sterilize 32 times, and those of iron (ferrous), copper, and manganese, 13 times their weight; lesser quantities, however, might be effective in practice, because the larvae from cultures so treated die comparatively rapidly after reaching the third stage. Dry ferric sulphate will sterilize 20 times its weight. Sodium sulphide, sodium sulphite, and sodium sulphate are all so ineffective that only when applied dry or as strong solutions are they lethal; they sterilized from eight to five times their weight. Magnesium sulphate will sterilize only four times and zinc sulphide twice its weight of fresh faeces. Ferrous sulphide and flowers of sulphur do not appear to have any lethal value, but the latter may, by controlling antagonistic factors, increase the chances of survival of the larvae.

Introduction

This paper reports on the lethal properties, against the free-living stages of Sclerostomes in fresh faeces, of some chemicals containing sulphur.

Potassium xanthogenate and carbon disulphide were tested because they have, under some conditions, proved effective against plant nematodes. Cupric sulphate was tested because it has for many years been suggested for treating pastures to kill nematode eggs and larvae, is used on pastures to kill snails, and is the standard chemical to use as an anthelmintic against some adult bursate nematodes. Flowers of sulphur was tested because it is the standard lethal agent against certain pests. Zinc sulphate, ferric sulphate, ferrous sulphate, manganous sulphate, sodium sulphide, sodium sulphite, sodium sulphate, magnesium sulphate, and ferrous sulphide were selected for testing because they are salts of the more important minor elements necessary for plant growth and for comparison with the chlorine salts of the

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[2] *Lecturer in Parasitology.*

same metals (to be discussed in the next paper), and also because it has been shown that sulphur and chlorine in some chemicals can exert important influences on the sheath and on the exsheathment of larvae when free of faeces (21, 22, 23). For instance, some sulphides may alter the permeability of the sheath and might be of considerable importance in bringing the lethal chemicals into contact with the larvae. It has been shown that some sulphur compounds are more effective than analogous oxygen compounds against mosquito larvae, with the exception of diphenylene oxide (3). Ammonium sulphate (32), potassium sulphate (33), and nicotine sulphate (34) have been discussed in previous papers.

Copper sulphate has been tested relatively frequently against the free-living stages of bursate nematodes, especially in Germany. Some of the more recent papers include data comparable with those in this paper. In 1930 it was reported that a 1% solution rapidly made third-stage Sclerostome larvae motionless, and that even a 0.02% solution had this effect in a few hours; furthermore, on being removed to water they did not recover (29), a characteristic effect of copper sulphate, which had been previously noted (1). However, it was shown, about the same time, that against Sclerostome eggs and third-stage larvae in faeces, 1 and 2% solutions were not satisfactory (38). In 1934 it was reported that 1, 0.5, and 0.1% solutions were lethal to third-stage Sclerostome larvae in 15 to 30 hr., but that a 1% solution did not prevent the eggs maturing to the third stage, which a 2% solution did prevent. It was also reported that when a 0.1% solution was used on grass, about 50% of the larvae survived, but only 3 to 8% survived when a 1% solution was applied (12). It has since been stated that the addition of copper sulphate to kainit adds to the efficiency of the latter, and that under laboratory conditions a 4 : 1 kainit: copper sulphate mixture at the rate of about 300 lb. per acre killed all the eggs, but that out of doors the results were less promising (24).

Copper sulphate has also been tested against the eggs and larvae of *Stephanurus dentatus*. A 1 : 1,000 solution killed both free-feeding and infective larvae in 24 hr., and stronger solutions were more rapidly lethal. However, against infective larvae in mud, a 1 : 10 solution took 72 hr. to be effective when applied at the rate of 10 gal. per 100 sq. yd. (39), whereas a 1% solution at the rate of approximately 4 gal. per 100 sq. yd., although frequently applied, was not effective against either *Stephanurus* or *Oesophagostomum* larvae (40). A 12½% copper sulphate solution has been tested for 30 min. against *Anguillulina dipsaci* in sweet potatoes (20), and for two hours, as a 2, 1, and 0.5 solution against *Tylenchus (=Anguillulina) dipsaci;* in two hours the 2% solution was lethal, but not the weaker solutions (28). Under similar conditions, 2% solutions of ferrous sulphate, sodium sulphate, and sodium sulphite were not lethal (28). Copper sulphate and sodium sulphite were not lethal (28). Copper sulphate has also been reported as useless against *Heterodera schachtii* (4).

Copper sulphate applied to manure at the rate of one-quarter pound and one pound in one gallon (American) of water per cubic foot of manure killed about 60% of fly larvae; iron sulphate was not effective (6). Iron sulphate has been tested less frequently than copper sulphate against nematodes. As a 1% solution it did not prevent hookworm eggs from hatching, but killed the free-feeding larvae (35). At the rate of 1680 lb. per acre, ferrous sulphate did not control the cysts of the *Heterodera schachtii* (10, 11), but in other small scale experiments ferrous sulphate did control potato sickness caused by *H. schachtii*, although less rapidly than did ferric chloride (18).

A few other sulphur salts, such as magnesium sulphate, have been tested against various pests. Magnesium sulphate was reported to kill wireworm *(Agriotes mancus)* larvae in soil (17), but not to kill fly larvae in manure (7). Sodium sulphide as a 0.6% solution was stated to be effective against *Tylenchus dipsaci* in two hours (27).

Sulphur has frequently been tested for the control of plant nematodes; the results, like those with so many other chemicals, are difficult to compare and are contradictory. It has been stated that 400 to 600 lb. of powdered sulphur per acre, prevented root-knot of sweet potatoes, and that even 200 lb. was beneficial (36); 250 to 500 lb. per acre has also been recommended against root-knot (42). Sulphur, at the rate of one pound per bushel of manure, was effective against fly larvae (5). Mixed with formaldehyde, it was reported as being more effective than lime against *Heterodera marioni* (43), but alone was useless against *H. marioni* (2), and against *H. schachtii* (19, 25), even with dressings of 500 to 1000 lb. per acre (37). In greenhouses, although applied at the rate of 2600 lb. per acre, sulphur gave no benefit against root-knot (26).

Carbon disulphide and potassium xanthogenate (which on decomposition releases carbon disulphide believed to be the cause of the latter's toxicity (30)) have also frequently been tested against plant nematodes. Again the reports show how varied the results have been. In pot experiments, carbon disulphide was stated to be of no value (4). At a rate of 220 gal. (American) per acre, carbon disulphide was also reported to be of no benefit in greenhouses (26), and at 30 gal. did not affect materially the degree of infection of potatoes with nematode disease (9). It was, however, stated to be superior to calcium cyanide, calcium cyanamide, and quicklime (8), but less effective than formaldehyde (43). Applied as an emulsion, containing 68% carbon disulphide, further diluted with 50 parts of water to one of emulsion, one gal. (American) per sq. ft. was reported to be effective against *H. marioni* (14). At the rate of four pounds per 100 cu. ft. of soil, when the temperature was 77° F., carbon disulphide was completely effective against *Rhabditis lambdiensis*, and two and a half to three pounds per cubic foot killed most of those mushroom parasites (16).

In pots, carbon disulphide also reduced the number of cysts on potatoes (25). At the rate of 750 lb. per acre it decreased nematodes by 48% (13), while 13.0, 20.0, and 30.0 cc. reduced *H. marioni* in 12 kg. of sandy soil to

6.4, 5.2, and 0.61% respectively (15). In practice, it was reported to give satisfactory results (42), especially against nematodes on trees and shrubs (41).

Potassium xanthogenate has been shown to be more effective against *H. radicicola* than against *H. schachtii;* larvae of the former are killed by 0.35 gm. per 300 cc. (=2750 lb. per acre foot) in light soil (30). The vapours of potassium xanthogenate were reported to penetrate soil and paralyse larvae in three days, and in small-scale experiments, at the rate of 140 lb. per acre, to have controlled potato sickness (18).

Methods

Full descriptions of the technique used to obtain the data on the lethal values of chemicals against the free-living stages of Sclerostomes have been given in earlier papers (31, 33). The results obtained with the chemicals discussed in this paper were all obtained by the same methods. Cultures of 40 gm. of *fresh* (less than four or five hours old) horse faeces, were treated with the chemical to be tested, and left to develop in a dark constant temperture room at 26° C. ± 3° C. for several days or weeks; the larvae were then extracted in funnels, from which they were drawn on the tenth day. This technique probably gives a very exacting test of the chemical, since the conditions are very suitable for rapid development of the larvae. However, some larvae may escape contact poisons by crawling up the walls of the glass container, or gases by remaining in small lumps of faeces. When the larvae reach the infective stage and then die, it is possible to recover only those on the outside of the culture or on the walls of the container.

Exact data are not available, but the sediment visible in the tap water used during the extraction process suggested that the quantity of impurities increased during the latter part of this work. These impurities occasionally amounted to about 0.01 gm. per 500 cc., chiefly consisting of iron carbonate.

The 9-in. extraction funnels held about 1600 cc., and approximately the same quantity is poured off and replaced during the extraction process. However, it is probable that the concentration of impurities was insufficient to affect the larvae, either by making them exsheath or otherwise injuring them.

Results

Potassium Xanthogenate

Potassium xanthogenate was tested dry and in solution; the results obtained are illustrated in Fig. 1.

It was tested dry in quantities ranging from 0.25 to 20% of the faeces by weight. The addition of 0.2 to 0.5 gm. considerably reduced the number of larvae. However, from some of the cultures to which 1.0 gm. or more was added, a few larvae, many dead, were recovered; from one of the cultures treated with 0.75 gm., equivalent to 1.87%, a few larvae were obtained; the other two cultures were sterilized.

A 1 : 2 aqueous solution was applied in quantities of 0.25 to 15.0 cc.; the addition of 1.5 to 2.0 cc. reduced the number of larvae recovered to a

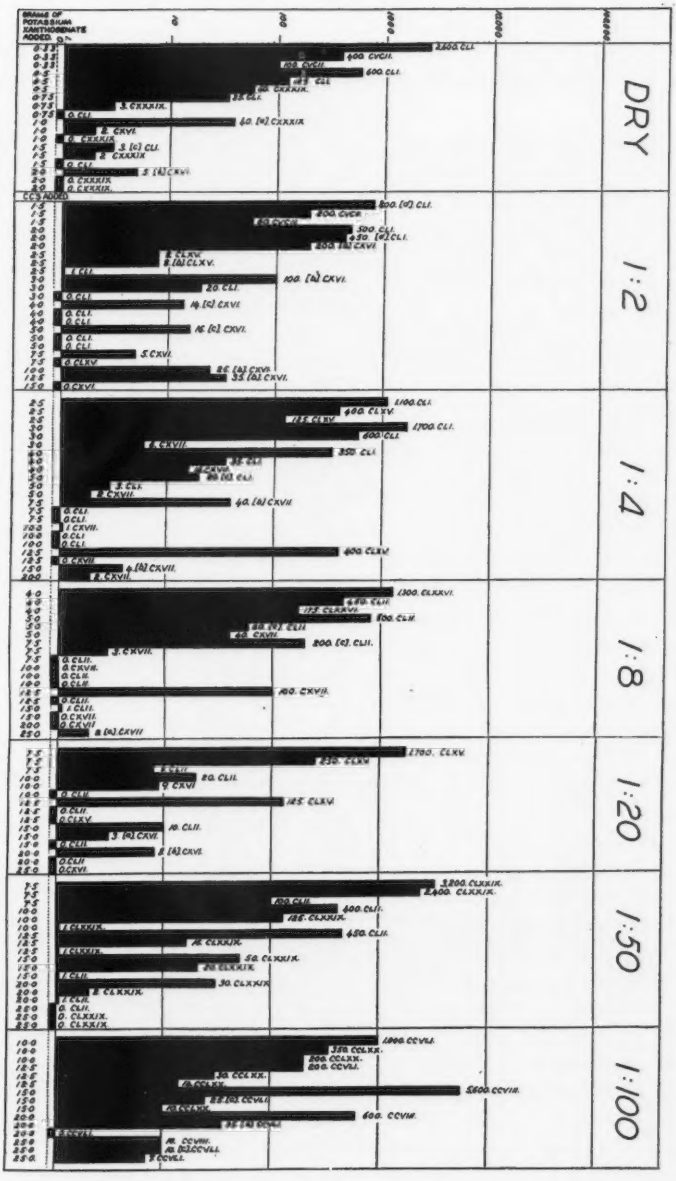
GRAMS OF POTASSIUM XANTHOGENATE ADDED
DRY
1:2
1:4
1:8
1:20
1:50
1:100

FIG. 1.

few hundred, and 2.5 cc. almost sterilized the cultures. However, from some of the cultures treated with 3.0 cc., and more, a few larvae, many of which were dead, were recovered. In 2.5 cc. of a 1 : 2 solution, there is almost 1.0 gm. of potassium xanthogenate, or 2.5% of the weight of the faeces.

The results obtained with a 1 : 4 solution, which was added in quantities of 0.5 to 20.0 cc., were also extremely irregular, but they suggest that 5.0 cc. will cause sterilization, although 400 larvae were recovered from one culture treated with 12.5 cc. In 5.0 cc. of solution of this strength there is slightly more than 1.0 gm., equivalent to 2.75% of the weight of faeces.

All the cultures receiving with 3.0 cc., or less, of a 1 : 8 solution yielded thousands of larvae. From two cultures treated with 5.0 cc. only 60 and 40 larvae were obtained; two of three cultures were sterilized by 7.5 cc. and all three cultures by 10.0 cc. One hundred larvae were recovered from one culture treated with 12.5 cc. In 10.0 cc. of a 1 : 8 solution there is also slightly more than 1.1 gm.

As a 1 : 20 solution, 2.0 cc. was the smallest quantity added. The results were again irregular; one culture was almost sterilized by 7.5 cc., 10.0 cc. was almost effective in three cultures, but 125 larvae were recovered from one receiving 12.5 cc. In 10.0 cc. of a 1 : 20 aqueous solution there is only slightly less than 0.5 gm., or 1.25% of the weight of the faeces in the cultures.

With one exception, all the cultures treated with not more than 7.5 cc. of a 1 : 50 aqueous solution contained thousands of active Sclerostome larvae. The addition of 10.0 cc., or more, considerably reduced the number of larvae in all cases. If the results, which were again irregular, are averaged, they suggest about 15.0 cc., containing 0.3 gm., equivalent to only 0.75%, as the approximate quantity necessary to sterilize the faeces.

A 1 : 100 aqueous solution was added in quantities ranging from 3.0 to 25.0 cc. All cultures receiving 5.0 cc., or less, yielded many thousand larvae. In two cultures, 7.5 cc. reduced the larvae to 700 and 550; in two, 10.0 cc., reduced them to 350 and 200. Those treated with 12.5 cc. yielded only 200, 30 and 14, but from one culture with 15.0 cc., 5600 larvae were isolated, and 600 from a culture treated with 20.0 cc. The other cultures receiving 15.0 and 20.0 cc. were practically sterilized, as were three to which had been added 25.0 cc., which contains about 0.25 gm., or 0.63% of the weight of the faeces.

Potassium xanthogenate was also tested as a 1 : 200, as a 1 : 300, and as a 1 : 400 aqueous solution; the results obtained are not illustrated. The 1 : 200 solution was added in quantities of 5.0 to 25.00 cc. All the cultures contained thousands of larvae with the exception of one treated with 20.0 cc. which contained 40, and those with 25.0 cc., which contained 500, 75, and 40 larvae.

One culture to which was added 25.0 cc. of a 1 : 300 solution contained only one larva, but the other cultures treated with a 1 : 300 solution and all treated with a 1 : 400 solution contained thousands.

These results indicate that potassium xanthogenate is most effective when applied as a weak solution; this seems to suggest that it may act at least partially as a contact poison, or, if its lethal property is in the gas that it liberates, that the penetrating power of the gas is extremely low.

Carbon Disulphide

Carbon disulphide was tested in quantities ranging from 0.025 to 25.0 cc. Fig. 2 illustrates how irregular were the results obtained. It is improbable that this irregularity could be ascribed to loss of the chemical from the containers as a gas, especially in those cultures that received the largest quantities as there was no odour of the gas in the constant temperature room. It can more probably be attributed to eggs or larvae or both in small lumps of faeces escaping its action.

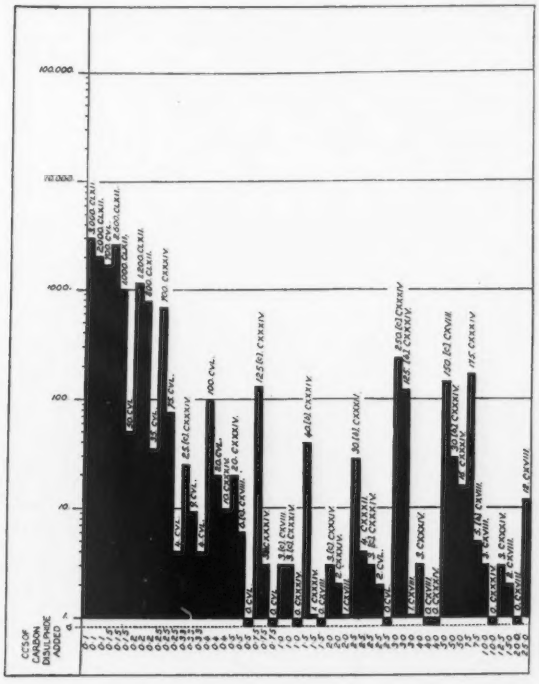

FIG. 2.

Even small quantities of carbon disulphide considerably reduced the number of larvae in most cases. The three cultures treated with 0.33 cc. were practically sterilized, as were a few with lesser amounts, but from two of the cultures receiving 3.0 cc., 250 and 175 larvae were obtained, including some dead, and from cultures treated with 5.0 and 7.5 cc., 150 and 175 larvae

were obtained. These results suggest that approximately 1.0 cc. will cause sterilization under conditions comparable with the technique used. If carbon disulphide gas is the lethal form against Sclerostome larvae, it is probable that, in containers filled by faeces, and if the gas would penetrate the faeces satisfactorily, it would be relatively more effective. However, the irregular results both with potassium xanthogenate and with carbon disulphide suggest that the characteristic of penetrating faeces, which would be so essential in practice, is lacking. The addition of 1.0 cc. of carbon disulphide is equivalent to adding approximately 3.1% of the chemical, by weight.

Zinc Sulphate

The results obtained with zinc sulphate are illustrated in Fig. 3.

This chemical, like other sulphur or chlorine salts of copper, iron, zinc, etc., frequently causes the death of the larvae after they have reached the third stage, although added to the faeces before eggs could have made much development. Whether these larvae, even soon after reaching the third stage, would have been capable of infecting a host is not known.

Zinc sulphate was mixed dry in cultures in quantities of 0.25 to 8.0 gm. When 0.5 gm., or less, was added, numerous larvae, although fewer than in the controls, reached the third stage and continued to live. When 0.75 to 1.5 gm. was mixed in the culture, many larvae reached the third stage, but a considerable proportion died comparatively rapidly. In one out of three cultures treated with 2.0 gm., numerous larvae reached the third stage but died subsequently; similar, but less marked, results were obtained when 3.0, 4.0, and 5.0 gm. were added. An average of these results suggests that about 2.5 gm., equivalent to 6.25%, is the amount of dry zinc sulphate necessary to cause sterilization.

Applied as a 1 : 2 aqueous solution 0.5 cc., or less, caused a comparatively slight reduction in the number of larvae. When from 0.75 to 4.0 cc. was added, numerous larvae were still isolated, but in most cultures many of these rapidly died. The addition of 5.0 cc. limited the number of larvae and also impaired their vitality. From one of three cultures treated with 7.5 cc. many larvae were isolated, but most of these soon died. The addition of 10.0 cc., or more, was effective in causing sterilization. In 7.5 cc. there is approximately 3.0 gm. of this chemical or 7.5% of the weight of the treated cultures.

When 5.0 cc., or less, of a 1 : 4 solution was added, numerous larvae were recovered from all the cultures; when 2.5 cc., or less, was added, the vitality of the larvae did not appear to be affected, but the addition of 3.0 cc., or more, had a marked effect on their viability. One hundred or more larvae were recovered from one of three cultures treated with 7.5, 15.0, and 20.0 cc., but many were dead. In 10.0 cc. of this solution there is about 2.25 gm. of zinc sulphate, or approximately 5.6% by weight.

Less than 5.0 cc. of a 1 : 8 solution did not have much effect on the vitality of the numerous larvae recovered; the addition of 7.5 cc., and more, reduced

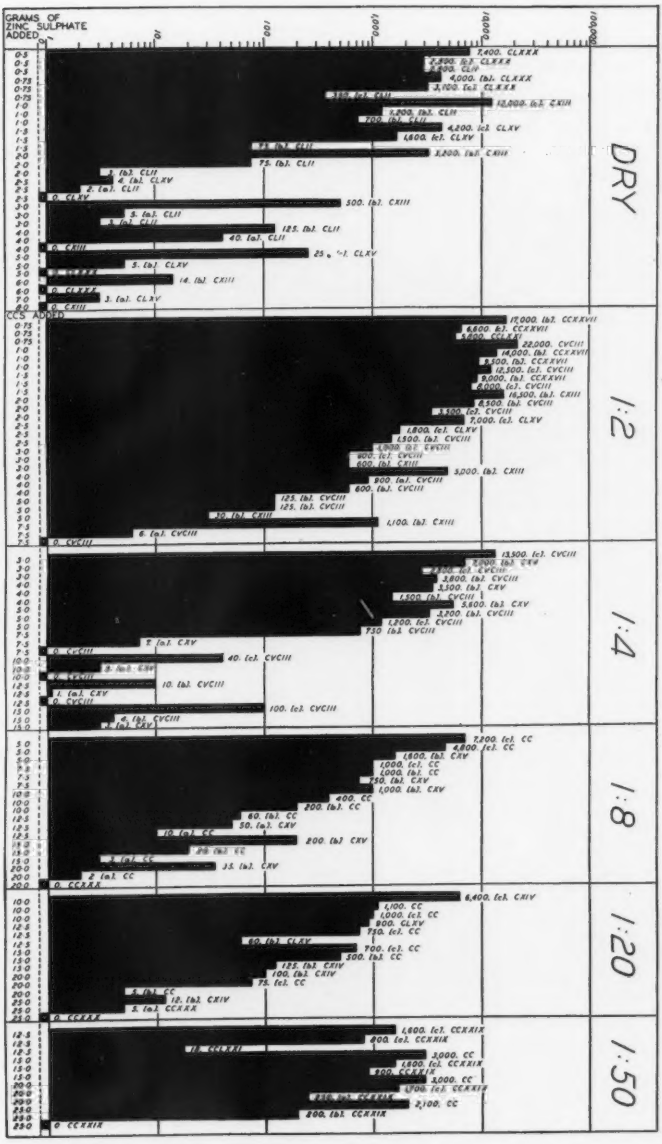
GRAMS OF ZINC SULPHATE ADDED
0·1
0·01
0·001
0·0001
0·00001
DRY
1:2
1:4
1:8
1:20
1:50

FIG. 3.

their numbers and viability. An average of the results obtained suggests that about 15.0 cc. of a 1 : 8 solution is necessary to cause sterilization. In this quantity there is about 1.8 gm., or 4.5% by weight.

As a 1 : 20 aqueous solution, 10.0 cc. was the minimum quantity that had a consistently marked affect on the numbers and vitality of larvae. The addition of 12.5, 15.0, and 20.0 cc. progressively reduced the numbers, but 25.0 cc., containing slightly less than 1.25 gm., equivalent to 3.1% by weight, is probably necessary to cause complete sterilization.

The results obtained with a 1 : 50 solution, which was tested in quantities ranging from 4.0 to 25.0 cc., were very irregular, both as to numbers and the viability of the larvae. One culture was completely, and another almost, sterilized by 25.0 cc., but from the third, 2100 active larvae were isolated. Smaller quantities of this solution, even 10.0 cc. or less, reduced the numbers of the larvae.

The fact that zinc sulphate is less effective dry, or as a strong solution, seems to suggest that to act on the larvae it must be in very intimate contact with them. Since many survive for some days or weeks after reaching the third stage and then die, it may also be suggested that the poison can penetrate

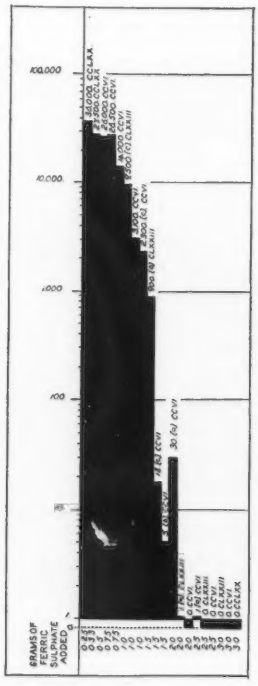

FIG. 4.

the sheath only slowly, and as third stage larvae do not feed in the free stage, it also suggests that it may not be necessary for the larvae to ingest the chemical to be killed by it.

Ferric Sulphate

Ferric sulphate was tested dry only; the results are illustrated in Fig. 4.

Many thousands of active larvae were recovered from the cultures receiving with 0.75 gm. or less. The addition of 1.0 gm. caused a slight reduction, whereas 1.5 gm. caused a considerable reduction in both numbers and viability of the larvae. When 2.0 gm., or 5%, was added no live larvae were recovered. One dead larva was recovered from a culture treated with 2.5 gm., but all the other cultures, treated with up to 8.0 gm., were free of larvae.

Ferrous Sulphate

Fig. 5 illustrates the results obtained with ferrous sulphate.

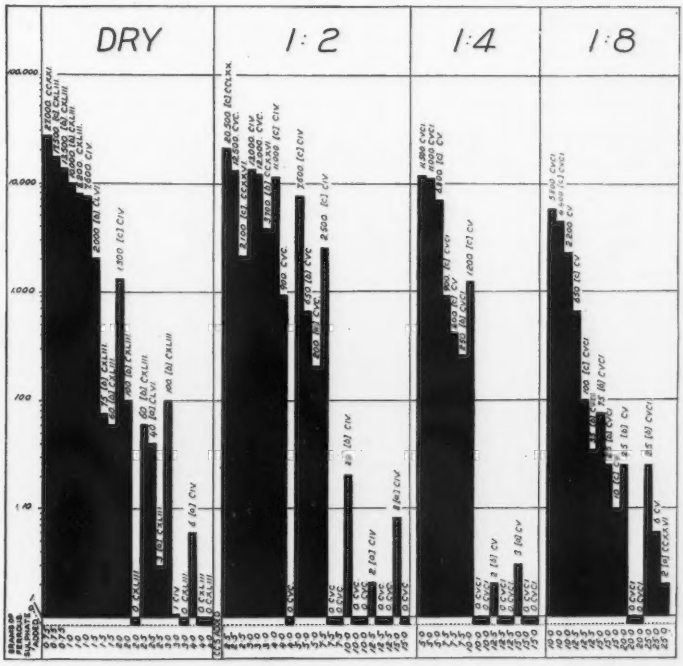

FIG. 5.

This chemical was applied dry in quantities ranging from 0.25 to 8.0 gm. Several thousand larvae were recovered from all cultures treated with 1.0 gm., or less, but some of the larvae obtained from one culture with 0.33 and with 1.0 gm., and from two with 0.75 gm., were dead. The addition of

larger quantities markedly affected the number and viability of larvae. Only from one of three cultures receiving 1.5 and 2.0 gm. were more than a thousand larvae obtained. All other cultures treated with 1.5 gm., and more, yielded only 100, or fewer, larvae—most, or all of which, were dead. An average of these results suggests that at most 3.0 gm., equivalent to 7.5%, is the quantity necessary to effect sterilization.

From one of three cultures treated with 7.5, 5.0, and 4.0 cc., and from all cultures with 3.0 cc., or less, of a 1 : 2 solution, thousands of larvae were isolated; however, some of those from some cultures receiving 2.5 cc., or more, were dead. All larvae from one culture treated with 5.0 cc., were dead, one with 4.0 cc. was free of larvae, as were two with 7.5 cc. The addition of 10.0 cc., and more, sterilized the cultures. This quantity of fluid contains approximately 4.0 gm., or 10% by weight, of the treated faeces.

A 1 : 4 solution was tested in quantities ranging from 2.0 to 25.0 cc. All cultures containing 5.0 cc., or less, yielded thousands of active larvae, with the exception of one treated with 5.0 cc., which yielded a few dead larvae. Three cultures treated with 7.5 cc. and one of the cultures with 10.0 cc. yielded many larvae, but some in each were dead. The others receiving 10.0 cc., or more, were sterilized. In 12.5 cc. of this solution there is almost 3.0 gm. of iron sulphate, or 7.5% by weight.

Applied as a 1 : 8 solution, thousands of larvae were recovered from all cultures to which 10.0 cc., or less, was added, and the vitality of most larvae seemed unaffected. When larger quantities were mixed in the faeces, both numbers and viability were markedly reduced; fewer than 100 larvae were recovered from one of the cultures treated with 12.5 cc. and from all receiving larger quantities. Two cultures were completely sterilized by 20.0 cc. and only a few larvae recovered from the third, as well as from the three treated with 25.0 cc.; most of these larvae were dead. In 25.0 cc. of this solution there is about 3.0 gm.

The results obtained by adding a 1 : 20 solution are not illustrated. It was tested in quantities of 5.0 to 25.0 cc. From all cultures, with the exception of one treated with 20.0 cc. and one with 25.0 cc., thousands of larvae were isolated; even from the two exceptions, 450 and 900 larvae were obtained. In the other cultures the vitality of only a few larvae appeared to have been lowered.

Cupric Sulphate

The results with cupric sulphate are illustrated in Fig. 6.

Very considerable differences exist in the quantities of this chemical necessary to free cultures of larvae and to cause many of the larvae to die after reaching the third stage. This makes an exact interpretation of the results more than usually difficult.

Dry cupric sulphate was added to cultures in quantities ranging from 0.25 to 10.0 gm. The vitality of the larvae in two of three cultures treated with 0.75 and with 1.0 gm., was affected, more especially with the larger quantity.

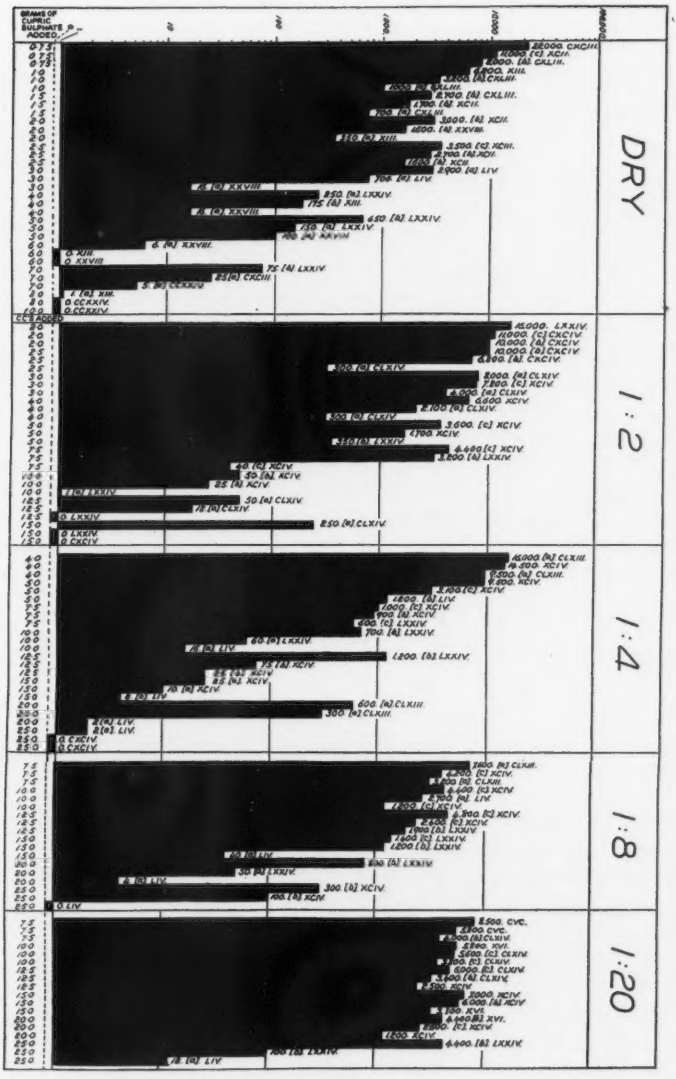

FIG. 6.

When 1.5 gm., or more, was added, some, or all, of the larvae from all the cultures were dead; yet some larvae were obtained from all cultures receiving 7.0 gm. or less, with the exception of two with 6.0 gm. All larvae were dead in one of three cultures treated with 1.0, with 1.5, and with 2.0 gm. and in two of three cultures with 4.0, with 5.0, and with 7.0 gm.; all the larvae

were dead, or the cultures free of larvae, when 3.0, 6.0, 8.0, or 10.0 gm. was added. On an average it is probable that about 4.0 gm., or 10% will kill the larvae, when added to fresh faeces.

A 1 : 2 aqueous solution was applied in quantities of 1.0 to 25.0 cc. A few of the numerous larvae recovered from one of the cultures treated with 1.5 cc. were dead. With the exception of one of three cultures receiving 2.0, 4.0, and 5.0 cc., all treated with 2.0 cc., and more, which yielded larvae, included dead larvae among them. In two of three cultures receiving 3.0 and 4.0 cc., the larvae were all dead; yet, in all cultures to which were added 5.0 and 7.5 cc., and in two of three with 10.0 cc., some were alive. No live larvae were found in any of the cultures treated with 12.5 cc. or more, whereas those treated with 20.0 and with 25.0 cc. were free of larvae. In 12.5 cc. of a 1 : 3 aqueous solution of cupric sulphate there is approximately 5.2 gm., or 13% of the weight of treated faeces.

When cupric sulphate was added as a 1 : 4 aqueous solution, 3.0 cc., or less, did not reduce the numbers or vitality of the larvae very considerably. From the cultures treated with 4.0 cc. many thousand larvae were recovered; however, those from two of the cultures were dead. The addition of 7.5 cc. considerably reduced both the number and viability of the larvae. The larvae from two of three cultures treated with 10.0 cc. were all dead, but a few were alive in all three receiving 12.5 cc. No larvae survived in any treated with 15.0 cc., or more; a few hundred dead larvae were recovered from two of three cultures treated with 20.0 cc. In 15.0 cc. of a 1 : 4 solution of copper sulphate there is slightly less than 3.5 gm., equivalent to approximately 8.7% by weight.

Added as a 1 : 8 solution, 4.0 cc., or less, did not affect the viability of the thousands of larvae recovered, and only a few were affected in two of the cultures treated with 5.0 cc. With a single exception over a thousand larvae were recovered from all cultures treated with 15.0 cc., or less; however, all the larvae were dead in one of three cultures receiving 10.0 and 15.0 cc., and in two of three with 7.5 and with 20.0 cc. In the others to which were added 7.5 cc., or more, there was a considerable loss of viability. An average of these results suggests that approximately 25.0 cc. of a 1 : 8 solution would be necessary to cause complete sterilization. In this quantity of solution there is 3.0 gm., equivalent to 7.5% of the weight of treated faeces.

When a 1 : 20 solution was applied to the faeces all cultures yielded thousands of larvae, with the exception of two of three cultures treated with 25.0 cc. Some of the larvae in about half of the cultures treated with 7.5 cc., or more, lacked viability. One of the cultures to which 25.0 cc. was added was sterilized; the larvae in one of the others were much reduced in numbers and viability, and many of the numerous larvae in the third also died. In 25.0 cc. of a 1 : 20 solution, there is slightly under 1.25 gm.

A 1 : 50 solution was also tested; thousands of larvae were recovered from all the cultures, but with the larger quantities a few of the larvae after reaching the third stage died. The results are not illustrated.

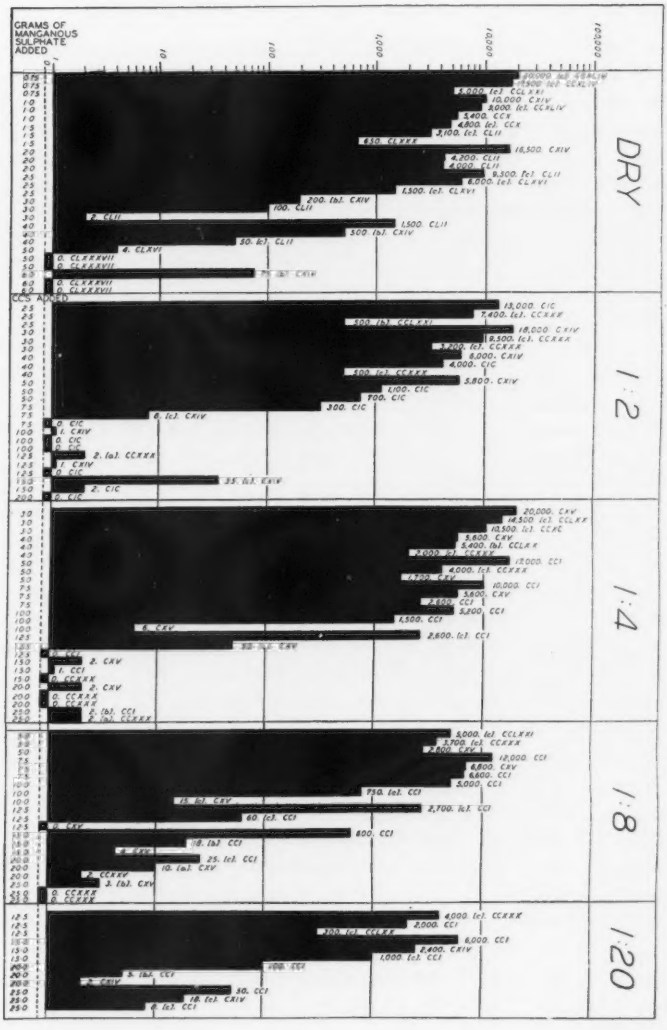

FIG. 7.

Manganous Sulphate

The effects of manganous sulphate are illustrated in Fig. 7.

Dry manganous sulphate was added to the cultures in quantities ranging from 0.25 to 10.0 gm. With the exception of one culture treated with 1.5 gm., which yielded 650 larvae, all those to which 2.5 gm. or less, was added yielded thousands of larvae; one culture to which 4.0 gm. was added also

yielded 1500 active larvae. A few of the larvae from all cultures treated with 0.75 and with 2.5 gm. were dead, as were a few from two of the cultures treated with 1.5 gm. and from one treated with 1.0 gm. The addition of 3.0 gm. markedly reduced the numbers and viability of the larvae, but the addition of 4.0 gm. was slightly less effective. Practically no larvae were recovered from the cultures to which 5.0 gm., equivalent to 12.5%, or more, was added, with the exception of one treated with 6.0 and one with 8.0 gm., from which 75 and 50 larvae were obtained, most of which were dead.

The minimum quantity of a 1 : 2 solution that was tested was 1.0 cc. Thousands of larvae were recovered from all the cultures treated with 5.0 cc., or less, with the exception of one with 2.5, 4.0, and 5.0 cc. However, some of the larvae in two cultures receiving 2.0 and 3.0 cc. and in one with 2.0 and 4.0 cc. were dead. One culture treated with 7.5 cc. yielded 300 active larvae, but the others with 7.5 cc., or more, were practically sterilized, with the exception of one treated with 15.0 cc. from which 35 larvae were obtained, of which a few were dead. These results suggest that, applied as a 1 : 2 solution, nearly 10.0 cc. containing over 4.0 gm., equivalent to about 10% is the necessary quantity to sterilize fresh faeces.

When added as a 1 : 4 solution, with the exception of one treated with 10.0 cc., which yielded only six larvae, all cultures treated with 10.0 cc., or less, and one treated with 12.5 cc., contained thousands of larvae; however, a few dead were included among those from one of the cultures treated with 2.5 and 5.0 cc., and from two treated with 3.0 and 4.0 cc. All cultures to which 15.0 cc., or more, and one to which 12.5 cc. was added, were practically sterilized. In 15.0 cc. of this solution there is nearly 3.5 gm. of manganous sulphate, or 8.75% of the weight of the treated faeces.

Applied as a 1 : 8 aqueous solution of manganous sulphate, 10.0 cc. was the minimum quantity that markedly reduced both the numbers and vitality of the larvae; in two of three cultures treated with this quantity some of the larvae were dead and their numbers were reduced to 750 and 15. The addition of 5.0 cc. also resulted in the death of some of the third stage larvae in two of the three cultures. One treated with 12.5 cc. was free of larvae, but, from the other two, 2700 and 60 larvae, including a few dead, were obtained. The addition of 15.0 cc. reduced the numbers in two of the cultures to 600 and 18, of which many were dead, and, in the third, to four. The cultures receiving 20.0 cc. were practically sterilized, and those with 25.0 cc. were completely sterilized; in the latter quantity there is about 3.0 gm., equivalent to 7.5%.

The addition of a 1 : 20 solution produced somewhat irregular results. From two of three cultures treated with 15.0 and with 12.5 cc. and from all treated with 10.0 cc., or less, thousands of larvae were obtained; however, from two treated with 12.5 cc. and from one treated with 10.0 and 15.0 cc., a few of the third stage larvae were dead. The addition of 20.0 cc. reduced the numbers to 100, 5 and 2; 25.0 cc. further lowered the numbers to 50, 18, and 8, including a few dead larvae.

Sodium Sulphide

Fig. 8 shows the results obtained with sodium sulphide. This chemical was tested dry in quantities ranging from 1.0 to 10.0 gm. The larvae in one of two cultures treated with 2.5 gm. were reduced in numbers and vitality, and 3.0 gm. had a more marked effect on the larvae of one of three cultures. The addition of 4.0 gm. reduced the larvae to 100, 50, and 14, and in the two last cultures, many were dead. When 5.0 gm., and more, was added, equivalent to 12.5% of the faeces by weight, the cultures were practically sterilized.

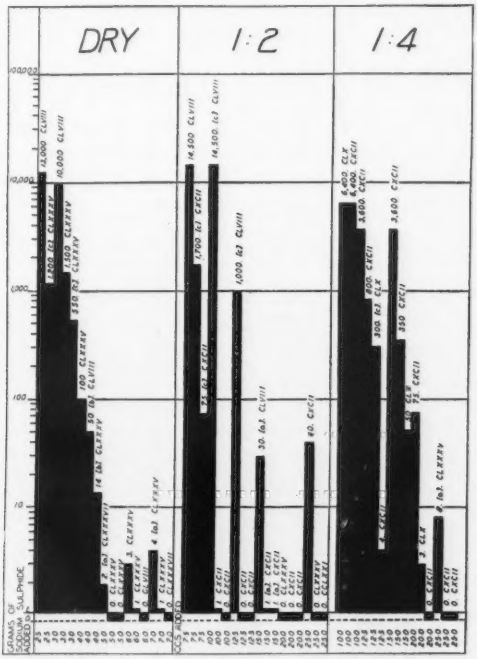

FIG. 8.

The larvae in one culture treated with 5.0 cc. of a 1 : 2 aqueous solution were reduced to 1200. In two of three treated with 7.5 cc. they were reduced to 1700 and 75, a few being dead in both cultures. In one of three cultures treated with 10.0 cc. the larvae were reduced to one, and another culture was free of larvae. From the other cultures treated with 10.0 cc., or less, many thousands of larvae were recovered. One culture receiving 12.5 cc. yielded nearly a thousand larvae, of which a few were dead, one receiving 25.0 cc. yielded 40 active larvae, but the other cultures treated with these, and the intermediate quantities, were sterilized. An average of these results

suggests that about 12.5 cc., which is also equivalent to about 5.0 gm., or 12.5%, is the lethal proportion.

With the exception of one culture to which 3.0 cc. was added, all the cultures treated with 10.0 cc., or less, of a 1 : 4 solution yielded many thousands of larvae. The comparative lack of larvae in the exception, from which only just over 800 were isolated, can probably be ascribed to the presence of the parasitic fungus, *Harpossporium anguillulae*, Lohde; in another culture, *Arthrobotryis obigospora*, Fres. was found, but it had not reduced the larvae to nearly the same extent, 15,000 being obtained.* The addition of 12.5 and 15.0 cc. of this solution considerably reduced the number of larvae. Two of three cultures treated with 20.0 cc. and all three treated with 25.0 cc. were sterilized. In the latter quantity there are approximately 5.5 gm., or 13.75% of the weight of faeces.

The results obtained with sodium sulphide as a 1 : 8 and as a 1 : 20 aqueous solution are not illustrated. With the exception of one culture treated with 20.0 cc. and of two with 25.0 cc. of the 1 : 8 solutions, and of one treated

**The writer is indebted to Dr. H. J. Brodie for the identification of these fungi.*

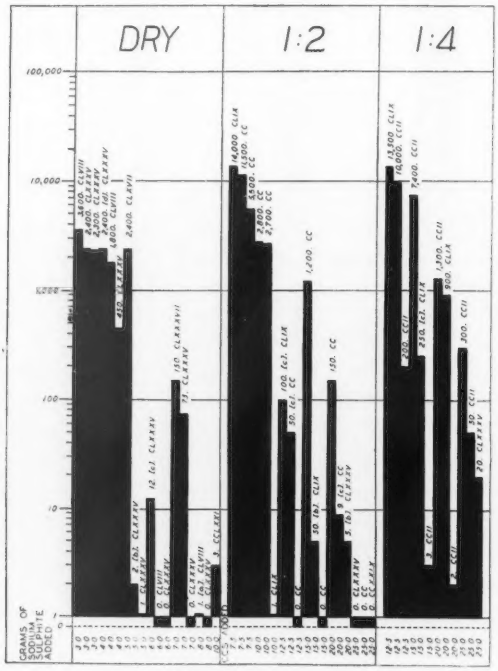

FIG. 9.

with 25.0 cc. of the 1 : 20 solution, all cultures yielded thousands of active larvae; from the exceptions, hundreds of active larvae were obtained.

Sodium Sulphite

The results obtained with sodium sulphite are illustrated in Fig. 9.

Thousands of active larvae were isolated from one of the three cultures treated with 5.0 gm., from two with 4.0 gm., and from all with 3.0 gm., or less. Two cultures receiving 7.0 gm. yielded 150 and 75 larvae, but the third culture was sterilized. Two treated with 5.0 gm., and all with 6.0, 8.0, and 10.0 gm., were practically sterilized. An average of these results suggests that 6.0 gm., or 15% is the lethal proportion.

One culture treated with 15.0 cc., two with 10.0 cc., and all with 7.5 cc., or less, of a 1 : 2 aqueous solution, yielded thousands of active larvae. One culture to which was added 20.0 cc. yielded 150 larvae; one receiving 12.5 cc. yielded 100 and another 50, which included dead larvae. The other cultures treated with these quantities were practically sterilized, one each by 10.0 and by 12.5 cc., two each by 15.0 and by 20.0 cc., and three by 25.0 cc. An average of the results indicated that almost 20.0 cc., containing 7.5 gm., equivalent to over 18%, is the approximate proportion to effect sterilization.

As a 1 : 4 solution, 12.5 cc. reduced the larvae in one culture to 200; 15.0 cc. reduced them in one culture to 250, including a few dead, and in another to three. The other cultures treated with these amounts, or with less, contained thousands of larvae. From the cultures treated with 20.0 cc., 1300, 900, and 2 larvae were obtained; 25.0 cc., which contains slightly under 6.0 gm. of sodium sulphite, reduced the larvae to 300, 50, and 20.

All cultures treated with a 1 : 8 and with a 1 : 20 solution, yielded many thousands of active larvae, with the exception of one of three cultures treated with 25.0 cc. of the 1 : 8 solution, which yielded only 20 larvae, and one of three treated with 25.0 cc. of the 1 : 20 solution, which yielded 1400 larvae, of which some were dead. The results with these solutions are not illustrated.

Sodium Sulphate

Fig. 10 illustrates the results obtained with sodium sulphate.

It was tested dry in quantities ranging from 1.0 to 14.0 gm. All cultures made with 4 gm., or less, contained thousands of active Sclerostome larvae. The addition of 6.0 and 7.0 gm. reduced the numbers of larvae and also the viability of a few of them. The numbers were further reduced in two cultures treated with 8.0 gm. and the third was free of larvae. One culture was completely and another almost sterilized by 10.0 gm., and most of the larvae obtained from the third were dead; this quantity is equivalent to 25% of the weight of faeces.

When a 1 : 2 solution was applied, one culture was sterilized by 15.0 cc. and all by 20.0 and by 25.0 cc.; those in which 15.0 cc. was used, and less, contained thousands of larvae. In 20.0 cc. of this solution of sodium sulphate, there is the equivalent of approximately 19% of the weight of faeces.

A 1 : 4, 1 : 8, and 1 : 20 aqueous solution were also tested; the results are not illustrated. The addition of 25.0 cc. of a 1 : 4 solution reduced one culture to 900 larvae, but all the other cultures made with this solution contained thousands of larvae.

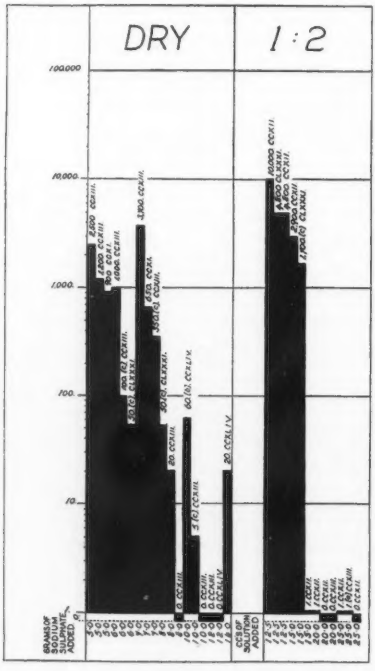

FIG. 10.

Magnesium Sulphate

The results obtained with magnesium sulphate are illustrated in Fig. 11.

Applied dry, all cultures treated with 6.0 gm. or less, contained thousands of active larvae; those treated with 7.0 and with 8.0 gm. also yielded many, although fewer active larvae. The addition of 10.0 and 12.0 gm. further reduced the numbers of larvae, while both quantities sterilized one culture. A few larvae were recovered from most of the cultures treated with 14.0 gm. (equivalent to 35% of the weight of the faeces), 16.0, and 20.0 gm., but all were dead, except those from one culture treated with 16.0 gm., which yielded 125 larvae.

All the cultures treated with 15.0 cc., or less, of a 1 : 2 solution yielded many thousands of larvae. Two cultures were sterilized by 20.0 cc. and two by 25.0 cc., but the third treated with 20.0 cc. yielded 350 larvae, some of

which were dead, and the third treated with 25.0 cc. (containing almost 10.0 gm. of magnesium sulphate, equivalent to 25%) yielded 2100 larvae.

A 1 : 4, 1 : 8, and 1 : 20 aqueous solution were also tested. Fewer larvae were found, of course, in the cultures treated with the largest quantities of fluid, but all the cultures contained thousands of active larvae. These results are not illustrated.

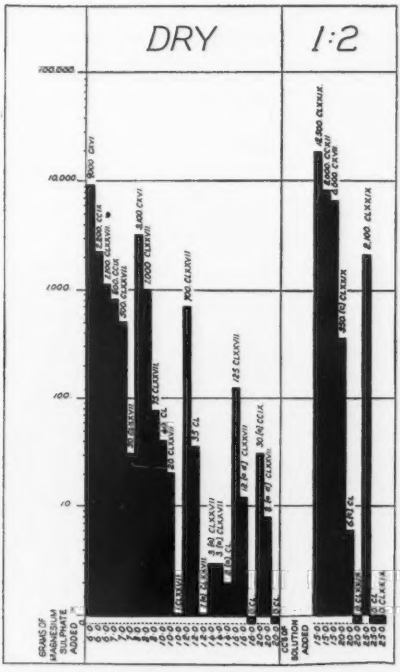

FIG. 11.

Zinc Sulphide

The results obtained with zinc sulphide which was tested dry only, are not illustrated.

All the cultures to which 10.0 gm. or less was added yielded many thousands of active larvae. The cultures treated with 12.0 and 14.0 gm. also yielded many thousands, most of them active, but in four of the five cultures, a few were dead. From one culture treated with 16.0 gm. only 100 larvae were obtained, many dead, but from the other two cultures treated with this amount, 41,000 and 2200 larvae were isolated, of which only a few were dead.

The addition of 20.0 gm. (50%) was more effective; one culture yielded only 30 dead larvae, another 200, of which a considerable proportion were dead, and the third 2200, including a few dead.

Ferrous Sulphide

Ferrous sulphide was tested dry only; it, like ferrous oxide, which was discussed in a previous paper (34), appears to have no lethal value. Ferrous sulphide was tested in quantities ranging from 1.0 to 20.0 gm. and compared with the controls there appeared to be no significant decrease or increase in the number of larvae isolated, or in their vitality.

Sulphur

Flowers of sulphur was tested in quantities ranging from 1.0 to 20.0 gm.; quantities of 10.0 gm., or less, were tested in duplicate, those over 10 gm., in triplicate.

TABLE I

CONTROLS FOR CULTURES TABULATED IN FIGS. 1–11

Series No.	Date culture made	Days kept in C.T. room	Average number of larvae isolated	Series No.	Date culture made	Days kept in C.T. room	Average number of larvae isolated
	1935				1936—*Conc.*		
XIII	7 May	31	11,000	CLXXIII	26 November	60	37,000
XVI	22	22	25,000	CLXXVI	5 December	52	71,000
XXIII	9 July	16	93,000	CLXXVII	5	58	33,000
XXIV	9	18	48,000	CLXXIX	8	58	98,000
XXVI	9	18	70,500	CLXXX	9	57	69,000
XXVIII	11	19	2,700	CLXXXI	9	57	72,000
LIV	10 December	17	6,200	CLXXXV	23	54	16,000
	1936			CLXXXVII	30	48	11,000
					1937		
LXXIV	24 January	24	11,500				
XCII	11 March	22	26,000	CXCII	8 January	55	12,000
XCIII	19	14	27,000	CXCIII	11	45	35,000
XCIV	19	21	35,000	CXCIV	12	51	31,000
CIV	7 April	23	21,500	CVC	12	55	22,500
CV	7	24	19,500	CVCI	19	48	29,500
CXIII	1 May	22	70,000	CVCII	20	47	31,000
CXIV	1	22	33,000	CVCIII	22	52	37,000
CXV	1	37	13,000	CIC	26	48	50,000
CXVI	7	15	36,000	CC	27	50	26,500
CXVII	7	23	26,000	CCI	28	56	40,000
CXVIII	12	21	2,000	CCII	29	48	19,000
CXXXII	12 June	22	8,500	CCVI	12 February	52	36,000
CXXXIV	17	37	40,000	CCVIII	18	49	55,000
CXXXIX	9 July	18	43,000	CCIX	18	46	40,000
CXLIII	31	74	18,500	CCX	23	44	35,000
CXLIV	31	77	13,500	CCXI	24	50	32,000
CVL	3 August	81	22,500	CCXII	1 March	38	51,000
CL	6	99	4,600	CCXIII	2	48	62,000
CLI	7	98	1,100	CCXXI	16	23	26,000
CLII	7	108	5,000	CCXXIV	17	65	41,000
CLVI	26 October	39	14,500	CCXXVI	30	48	34,000
CLVIII	29	43	39,000	CCXXVII	1 April	51	42,000
CLIX	30	44	71,000	CCXXIX	5	56	33,000
CLX	2 November	42	15,500	CCXXX	6	55	22,500
CLXII	5	49	39,000	CCXLIV	20 May	25	49,000
CLXIII	9	45	55,000	CCVLI	21	34	29,000
CLXIV	11	43	24,500	CCLXX	22 July	74	34,000
CLXV	12	53	43,000	CCLXXI	23	83	16,000
CLXVI	16	45	23,500	CCXC	28 October	28	46,000

Sulphur appears to have no value in reducing the numbers of larvae. In a few cultures made with various quantities, a few of the recovered larvae were dead, but there appeared to be no correlation between the amount applied and the subsequent death of the larvae. In fact, the results suggest that by controlling parasitic fungi or other harmful factors, the number of larvae recovered may be increased. In 20 of the 30 cultures more larvae were recovered from the treated than from the control cultures. The controls were those for series XIII, XXIII, XXVI, CXLIV, CLXV (see Table I). Five cultures contained more than three times as many larvae in the treated as in the control cultures, and ten, more than twice as many; naturally, the largest increases tended to be in the series with the fewest larvae in the controls—13,500 and 11,000. The average number of larvae in the treated cultures was 42,400, an increase of just over 25% of the number that the controls show, would have been in 30 untreated cultures.

Conclusions

Potassium xanthogenate, unless its cost were too high, might be used under some conditions as a lethal agent against the free-living stages of Sclerostomes, since it is fairly effective as a weak solution. Carbon disulphide, unless it proves more effective as an emulsion, does not appear to have sufficient penetrating power. The other salts discussed in this paper all seem too weak to be of value alone. The "delayed action" of some, however, suggests that, if they were brought into intimate contact with the larvae through the action of another chemical, they might have some value. Unfortunately, much more work must be done before any constructive proposals can be made on this basis.

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